

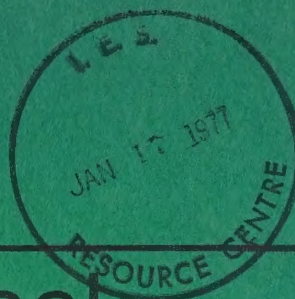
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A BACTERIOLOGICAL INVESTIGATION OF MEAT AND POULTRY
PACKING PLANT EFFLUENTS WITH PARTICULAR EMPHASIS ON
SALMONELLA. 1976.

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A Bacteriological Investigation of Meat and Poultry Packing Plant Effluents with Particular Emphasis on Salmonella

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Water Pollution Control Directorate
December, 1976

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A BACTERIOLOGICAL INVESTIGATION OF MEAT AND POULTRY PACKING
PLANT EFFLUENTS WITH PARTICULAR EMPHASIS ON SALMONELLA

Water Pollution Control Directorate
Environmental Protection Service
ENVIRONMENT CANADA

Report No. EPS 3-WP-76-9
December 1976

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PREFACE

The bacteriological quality of liquid effluents from meat and poultry products plants is becoming an increasing concern to regulatory agencies. Of particular concern are the levels of Salmonella in these effluents. Accordingly, the Environmental Protection Service of Environment Canada undertook two studies, one in Ontario and one in Alberta, in an attempt to quantify this problem.

This report, which represents the results of these two studies, indicates the lack of effectiveness of conventional treatment processes in the reduction of Salmonella populations. In addition, it describes the bacteriological characteristics of the raw effluents from meat and poultry packing plants. The data on which the conclusions and recommendations of these studies are based are limited, but provide sufficient indications that Salmonella may be a problem for all sectors of this industry. It has been recommended that disinfection of the treated effluents be practiced to control and limit the population of these organisms prior to discharge to the receiving environment.

PRÉFACE

Le contenu bactérien des effluents provenant des installations transformant les viandes rouges et la volaille et, plus particulièrement, leur taux de Salmonella, préoccupe de plus en plus les organismes de réglementation. C'est pourquoi le Service de la protection de l'environnement (Environnement Canada) a entrepris deux études, l'une en Ontario et l'autre en Alberta, afin d'obtenir des données quantitatives.

Le présent rapport, qui résume les résultats de ces études, signale que les procédés traditionnels de traitement ne sont guère efficaces contre la Salmonella. Il développe également les caractéristiques bactériennes des effluents non traités issus des usines de transformation des viandes. Les données sur lesquelles se fondent les conclusions et recommandations des deux études sont limitées, mais n'en indiquent par moins que la Salmonella peut créer des problèmes dans tous les secteurs de cette industrie. Le rapport recommande, entre autres, de désinfecter les effluents traités avant de les déverser dans la nature, afin de réduire et de limiter les populations de cette bactérie infectieuse.

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SALMONELLA IN POULTRY PACKING PLANT EFFLUENTS

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A BACTERIOLOGICAL INVESTIGATION OF MEAT PACKING PLANT
EFFLUENTS IN THE PROVINCE OF ALBERTA
WITH PARTICULAR EMPHASIS ON SALMONELLA

by

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Report No. EPS 4-NW-75-1
August, 1975



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ABSTRACT

The waste treatment facilities and final effluents of eleven meat packing plants in the Province of Alberta were investigated primarily to determine the numbers of indicator bacteria and the presence of *Salmonella*. This was done to discover the efficiency of the treatment systems presently in operation in reducing bacterial numbers, and to establish the need for disinfection and for bacterial standards for these effluents.

Data obtained showed that the final effluents were of very poor quality bacteriologically with numbers of indicator organisms comparable to those found in raw sewage. Primary treatment facilities were totally ineffective in reducing these bacterial numbers and in addition, *Salmonella* were isolated from the final effluents of 80% of the plants. Although secondary treatment was somewhat more efficient in reducing bacterial counts, again *Salmonella* were isolated from the final effluents. In total, 21 different serotypes were identified.

Salmonella isolates were not antibiotic resistant, but certain coliform and fecal coliform isolates demonstrated resistance to chloramphenicol, tetracycline, and ampicillin. *S. typhimurium* cells survived up to 12 days in nutrient-free water, and Rainbow Trout fingerlings became carriers of these organisms when placed in water containing an inoculum of the cells.

It is recommended that a bacterial standard be included in regulations controlling the quality of meat packing plants effluents and that disinfection be implemented.

RESUME

L'investigation des facilités touts-a-l'égouts et les cours d'eaux dérivés finals d'onze abattoirs dans la Province d'Alberta était mené à bien pour déterminer les quantités des bactéries indicatrices et la présence de *Salmonella*. C'était fait pour découvrir si les systèmes du traitement qui sont utilisés maintenant sont très efficaces pour réduire les quantités des bactéries, et, pour prouver le besoin de la désinfection et le besoin des règlements bactériens à l'égard des cours d'eaux dérivés.

Les resultats obtenus ont indiqués que les qualités bactériologiques des cours d'eaux dérivés finals étaient mauvais avec beaucoup d'organismes indicateurs qui sont comparable à ceux qui on trouve dans l'eaux d'égout. Les touts-à-l'égouts primitifs étaient complètement inefficaces pour réduire ces quantités des bactéries et outre cela, *Salmonella* s'étaient isolés des cours d'eaux dérivés finals de 80% des abattoirs. Bien que les touts-à-l'égouts secondaires étaient plus efficaces pour réduire les quantités des bactéries, *Salmonella* étaient encore isolés, des cours d'eaux dérivés finals. En somme, il y avait vingt-et-un sérotypes diverses qui sont reconnus.

Salmonella qui étaient dans l'isolement n'étaient pas résistant aux antibiotiques. Mais quelques-unes des coliforms et des coliforms fécales qui étaient dans l'isolement ont fait une résistance à Chloramphenicol, à Tetracycline, et, à Ampicillin. *S. typhimurium* cellules ont survécus depuis douze jours dans l'eau sans les nourritures et, les petites "Rainbow Trout" sont devenus les porteurs de ces organismes quand on les avait mis dans l'eau qui contenait une mass de ces cellules.

C'est une recommandation qu'un régulateur bactérien est très nécessaire dans les règlements dirigent les qualités des cours d'eaux dérivés finals des abattoirs et que la désinfection des abattoirs va exécuter et accomplir.

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CONCLUSIONS

1. Final effluents from the Alberta meat packing plants studied were of extremely poor quality bacteriologically. Numbers of indicator organisms (coliforms, fecal coliforms, fecal streptococci, and total heterotrophic bacteria) were in the hundreds of thousands to millions per ml, comparable to those found in raw sewage.
2. The primary treatment facilities were totally ineffective in reducing the indicator bacterial counts, and in some instances, numbers were higher in the effluents than in the influents. Influent temperatures of about 30°C and high concentrations of nutrients could conceivably support growth and multiplication of these organisms. In addition, even though secondary treatment was somewhat more efficient in reducing the counts, there still remained several thousands of the organisms per ml of final effluent.
3. Coliform and fecal coliform biochemical identifications showed that the membrane filtration procedures and specific isolation media were reliable techniques for the enumeration of these organisms in this particular type of waste. Therefore the use of the numbers of these organisms, for the characterization of these wastes, is valid.
4. *Salmonella* were isolated from the effluents of 7 of 9 meat and poultry packing plants, or, from about 80% of the final effluents investigated. In many instances, *Salmonella* were readily isolated from 10 ml and even as little as 1 ml aliquots of the effluents. Often, more than one serotype were found in the same effluent, for example, from one red meat packer in Edmonton, 11 serotypes were obtained from the final waste. Even after secondary treatment, in the Calgary plant with these facilities, 2 serotypes were obtained from the final effluent. In total, from 7 plants, 21 different serotypes were identified. Considering the limitations of methodology, the fact that many Moore swabs were "lost", and that in most cases, the plant effluents were sampled once only, this number of isolations and the variety of serotypes probably represent a gross underestimation of the number of *Salmonella* being released in these wastes, and possibly, of the extent of the frequency of carrier animals in Alberta livestock. It can reasonably be assumed that continued release of these organisms to the environment in non-disinfected wastes will increase their dissemination to animals and man.

5. The antibiotic resistance tests, on selected coliform and fecal coliform isolates and on all the *Salmonella* serotypes, revealed that although the *Salmonella* were sensitive to those antibiotics routinely used against Gram negative bacteria, a number of the coliforms and fecal coliforms were resistant to chloramphenicol and tetracycline. Of particular interest was the discovery that 56% of the coliform and 77% of the fecal coliform isolates were resistant to ampicillin, a drug commonly used against *E. coli*, *Klebsiella*, and other Gram negative organisms. These results further emphasize the possibilities and potential hazards of drug resistance transfer from resistant coliforms to receptive pathogenic bacteria in situations where these organisms are permitted to come into contact with each other, for instance in non-disinfected wastes.

6. *S. Typhimurium* cells survived at least 12 days in nutrient-free, 15°C water. This may indicate that salmonella, and perhaps, other bacterial pathogens, can survive for several days in water bodies such as streams, rivers, and lakes. Thus waters receiving such organisms become potential health hazards if used for public water supplies, irrigation, cattle watering, fishing, or for recreational purposes.

7. Rainbow trout fingerlings became carriers of *S. typhimurium* upon being introduced to water containing an inoculum of these cells. They continued to be carriers after having been removed and placed in fresh, uncontaminated water, even after a period of three days. This indicated that fish can be vectors of pathogen transfer to unpolluted waters. In addition, the release of *Salmonella* and other pathogens to waterways threatens the use of fish as a source of human food, and in the production of animal feedstuff.

RECOMMENDATIONS

1. The frequent isolations of *Salmonella* strongly suggest a need for further treatment of meat packing plant wastes, including disinfection.
2. The apparent effectiveness of secondary treatment in reducing bacterial counts should be further evaluated, and disinfection requirements established.

3. Regulations for meat packing plant wastes should include a bacterial standard. This standard could be established through the implementation of recommendation 2.

4. Assessments of the quality of meat packing wastes should be undertaken in other regions to establish the extent of the associated problems throughout Canada.

5. Where possible, the dissemination of *Salmonella* throughout the environment should be stopped and the Federal Department of the Environment should take lead role in combating this problem. Regulations prohibiting the discharge of these organisms in meat packing plant effluents are required.

1.0 INTRODUCTION

A preliminary investigation of the bacteriological quality of meat packing plant effluents in the Province of Alberta was instigated in response to a request from meat packing industry Task Force Co-ordinator, Mr. M.J. Riddle, Water Pollution Control Directorate. The project was designed to assess the occurrence and numbers of indicator organisms and *Salmonella* within the treatment systems and in the final effluents from a variety of meat packing industries throughout the Province. This was to provide information to serve as a basis for proposals concerning guidelines and standards for the bacteriological quality of the final effluents and possibly, the need for disinfection of these wastes.

The present study was conducted between October, 1974 and March, 1975. Three red meat, 1 horsemeat, and 2 poultry processing plants in Edmonton, 3 red meat plants in Red Deer, and 2 red meat plants in Calgary were investigated. Numbers of indicator organisms and the presence or absence of *Salmonella* within the plants' waste treatment facilities and in the final effluents were determined. This information enabled some estimations to be made regarding the efficiency of the primary or secondary treatment systems in reducing bacterial numbers and eliminating *Salmonella*, and was used as the basis for proposing disinfection of these wastes. Special emphasis was given to the study of the Calgary plant which has a secondary treatment system.

In each plant, basic design and operation were reviewed, and estimates of waste stream flows and gallonage of waste water produced daily were made. Optimum sampling points were selected in order to sample in-plant waste streams, separate discharges, the combined untreated waste effluent, and the final effluent.

The extent of cycling in the environment and the various methods of transmission of *Salmonella* were outlined in view of the fact that meat packing effluents containing these organisms are being released to municipal waste systems which do not include disinfection as part of the treatment program. The antibiotic resistance of several coliform, fecal coliform, and *Salmonella* isolates from final effluents were estimated and the possibilities and hazards of antibiotic resistance transfer among these bacteria were discussed,

again in relation to the lack of disinfection of these wastes.

Some *Salmonella* survival times in water were determined, and the potential role of fish as vectors of *Salmonella* considered.

2.0 LITERATURE REVIEW

Woollen (1974) stated that "salmonellosis is an increasing world problem", and that in spite of efforts at surveillance, incidents of salmonella infection are grossly unreported throughout the world. In England and Wales, human salmonellosis cases doubled between 1966 and 1971 (Lee, 1974) mainly due to a three-fold increase in the occurrence of salmonella serotypes other than *Salmonella typhimurium*. In the U.S.A. two million human cases and 500 deaths, and related economic losses of 100 million dollars annually, are attributed to salmonella infections (Prost and Riemann, 1967).

In Canada, the 5,000 cases diagnosed annually probably represent only about one percent of the total human salmonella infections. Thus in Canada, the number of persons afflicted per year with this condition may be closer to 500,000. In the Province of Alberta, there are 300 to 400 diagnosed cases of human salmonellosis annually (Finlayson, personal communication).

The increase in the number of reported salmonella outbreaks has resulted in intensified efforts to identify environmental vectors of salmonella transmission and recycling (Miner et al., 1967).

Salmonella serotypes are "zoonotic" meaning they are potential agents of disease in both animals and man. In man, theoretically, infection and disease can occur at any age. Most incidents, however, occur in infants and elderly persons, and depend upon social conditions and stress factors. The number of cells which constitute an infective dose depends upon both the virulence of the organism, and upon the physical condition and state of immunity of the host. Salmonellosis is a disease ranging in severity from mild gastroenteritis to septicemia, enteric fever, and meningitis possibly leading to death (Durrani, 1971).

2.1 Mechanisms of Salmonella Transport to Man

2.1.1 Food Poisoning. One of the major vehicles by which salmonella is transported to man is food. Food serves as a culture medium necessary for non-host adapted serotypes to reach numbers sufficient to cause infection and disease. Products which can serve in this capacity include red and white meats, unpasteurized eggs and egg products, milk, cream, pharmaceutical items made from animal substances, cereal grains, fruits, and vegetables. However, by far, the majority of salmonella food poisoning incidents are caused by infected meat and poultry products (Hobbs, 1974). Reports of epidemiological studies linking human food poisoning cases to salmonella-infected meat are available. Although these are too numerous to be catalogued and discussed in detail at the present time, a few are cited for reference purposes.

Salmonella-infected pork and pork products have been responsible for food poisoning in man (Linton et al., 1970; Ghosh, 1972; and Lee et al., 1972). A human infection outbreak of *S. typhimurium* in England was related to contaminated calf meat (Anderson et al., 1961). Poultry products also, have been vectors of human salmonellosis (Lee, 1974). Recently, an extensive work relating salmonella-contaminated food to food poisoning incidents has been compiled and edited by Hobbs and Christian (1973).

2.1.2 Animals to Man. Transmission of salmonella to man has been reported to occur from domestic pets, including dogs, cats, monkeys, rabbits, guinea pigs, rats, mice, turtles, birds, and fish, and from direct contact with livestock (Williams and Hobbs, 1974).

2.1.3 Man to Man. Man to man transmission of salmonella occurred where contact was made directly with human excretors, for instance in hospitals (Clarenburg et al., 1952; Rowe et al., 1969; MacGregor and Reinhart, 1973).

2.1.4 Contaminated Water Supplies. Human salmonella infections can be contracted from contaminated water used for recreation, drinking, food processing, or for the spray irrigation of vegetables and other food crops (George and Wallace, 1972; Anderson and Hobbs, 1973). Recently in North America, outbreaks of severe gastroenteritis and typhoid fever in Dade County,

Florida (Pfeiffer, 1973), in Detroit (Berger et al., 1963), and in the Province of Quebec, (Martineau and Breton, 1972), and the infection of eighteen thousand people in Riverside, California, with *S. typhimurium* (Geldreich, 1972), were found to have been the result of contaminated water supplies. In August 1975, more than 40 persons who had attended a religious camp near Kingston, Ontario, contracted typhoid possibly from contaminated drinking water.

2.2 Cycling of Salmonella in the Environment

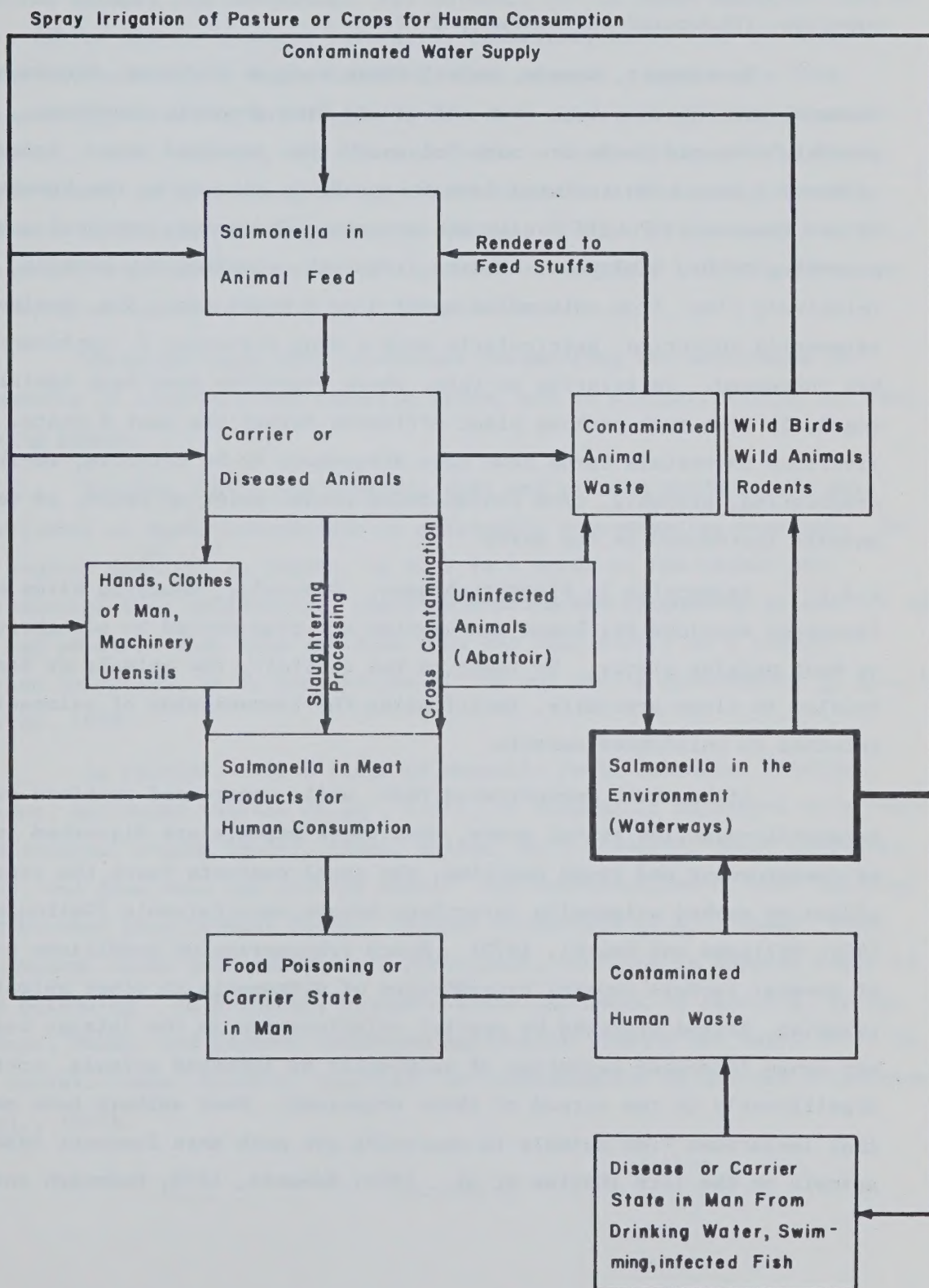
Some of the major routes by which salmonella can be cycled in the environment are shown in Figure 1. A principal direction of transmission is from feedstuffs to domestic animals to foodstuffs to man. Excrement from infected animals and humans, if released as untreated waste into waterways, can result in the further dissemination of salmonella if the water is used for drinking, or the spray irrigation of livestock pasture or crops for human consumption. Wild animals, birds, and fish, also, can become carriers and participate in the spread of these organisms.

2.2.1 Animal Feedstuffs. Contaminated animal feedstuffs can play a significant role as the origin of salmonella infections. Feeds thus continue to renew the cycles of salmonella transmission (Figure 1) even when these organisms have been eliminated from the intermediary steps.

Contaminated human food products and animal waste products are often rendered to animal feedstuffs. These feeds, such as fishmeal, bonemeal, soya, and dried and ground infected meat and vegetable wastes may be obtained from many parts of the world. Unfortunately, processing methods and legislation concerning such vary greatly from one country to another. In the U.K. and in several other countries, processed poultry manure is used in compound feeds. Conversely, in the U.S.A. this practice has been banned.

As the demand for fishmeal as a protein source has increased over the last few years, Peru has become the producer of 40% of the world's supply. Unfortunately it is commonly contaminated with salmonella and attempts to rectify this have not been successful. In addition, quality control sampling and testing are unsatisfactory (Clark et al., 1973).

FIGURE 1 PATHWAYS OF SALMONELLA TRANSMISSION



In Denmark, veterinary legislation requiring the sterilization of imported and home produced feedstuffs has reduced the incidence of salmonella in the feeds to 0.3% as compared to 20% to 30% incidence in other countries (Skovgaard and Nielsen, 1972).

In Alberta, Canada, animal feeds such as fishmeal, bloodmeal, and bonemeal are obtained both from abroad and from domestic suppliers. In general, domestic feeds are much "cleaner" than imported ones. However, salmonella have been isolated from feedstuff in Alberta by the Edmonton branch of the Provincial Public Health Laboratories (Finlayson, personal communication). According to Dr. Finlayson, Alberta livestock, particularly bovines, were relatively free from salmonella until 3 or 4 years ago. Now, bovine salmonella infection, particularly with a drug resistant *S. typhimurium*, has increased. In relation to this, these organisms have been isolated regularly from meat packing plant effluents during the past 4 years. The livestock on certain farms have been discovered to be carriers, their infections originating, probably, from contaminated feeds, drinking water, or carrier animals introduced to the herds.

2.2.2 Salmonella in Slaughterhouses. Generally, domestic birds and livestock destined for human consumption are transported to slaughterhouses or meat packing plants. En route to the abattoir, the animals or birds are huddled in close proximity, facilitating the transmission of salmonella from infected to uninfected animals.

It has been demonstrated that, while unstressed carriers produce salmonella-negative rectal swabs, when these animals are disturbed in transport by overcrowding and rough handling, the cecal contents reach the rectum, and hidden or masked salmonella infections become ascertainable (Galton et al., 1954; Williams and Newell, 1970). Hence transportation conditions can be one of several factors causing transmission of salmonella to other animals. Likewise, stress produced by spatial relationships in the lairage and abattoir may cause increased excretion of salmonella by infected animals, contributing significantly to the spread of these organisms. Many authors have emphasized that isolations from animals in abattoirs are much more frequent than from animals on the farm (Galton et al., 1954; Edwards, 1958; McDonagh and Smith,

1958; Takacs and Nagy, 1973; Hobbs, 1974).

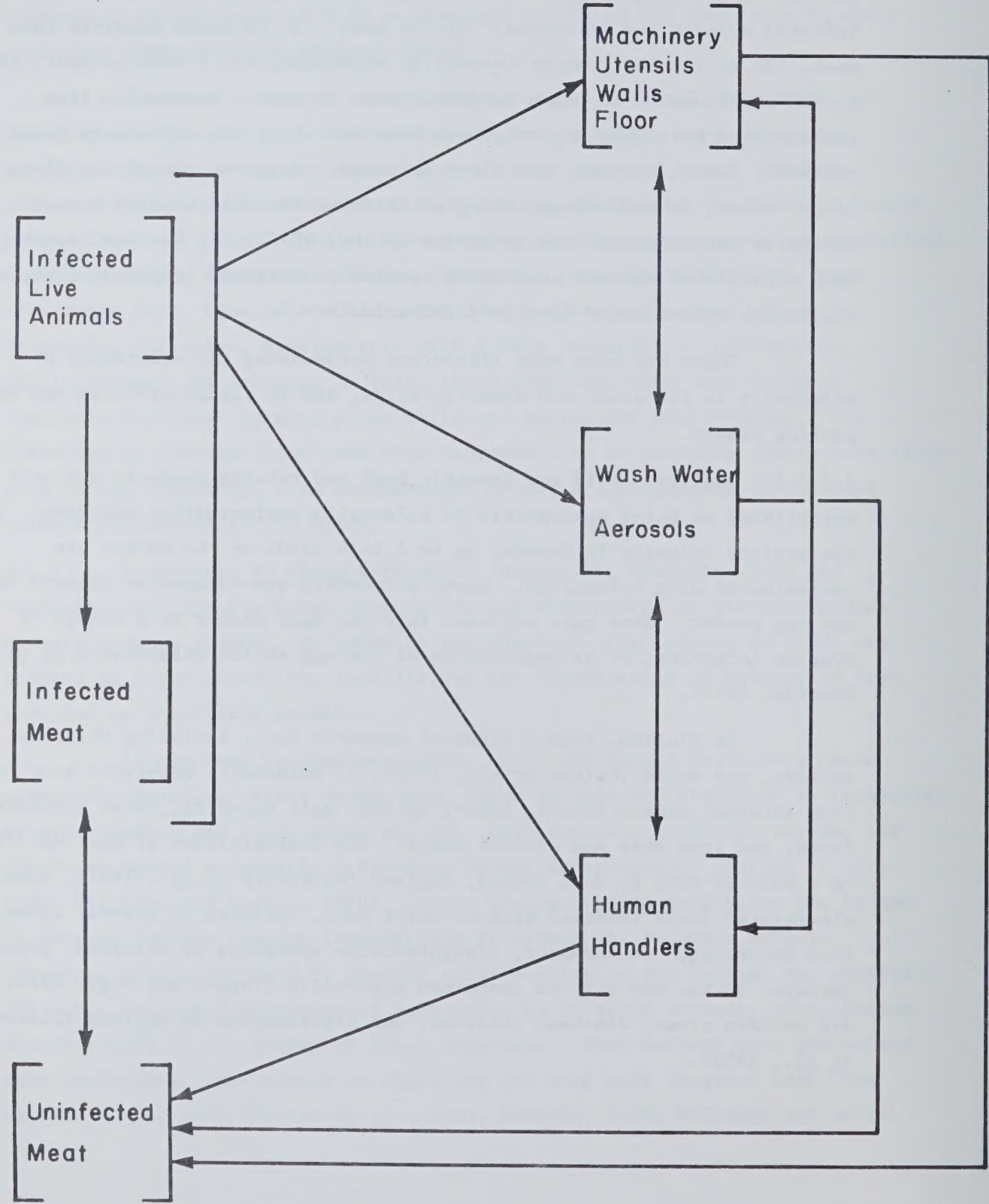
Within the packing plants, salmonella can be transferred by several routes (Figure 2). Principally, these transmissions are from infected animals and carcasses: (1) to meat, (2) to human handlers then to meat, (3) to the environment (utensils, washwater, etc.) then to meat, (4) to the environment, to human handlers, then to meat. Salmonella from contaminated carcasses may originate from the skin, the alimentary tract contents, feces, viscera, and blood aerosols. Examinations of the flora of gut rooms, in many slaughtering establishments, will usually reveal extensive environmental contamination (Hobbs, 1974). It has not, however, been established whether salmonella persist or multiply in these rooms, or are merely disseminated from infected animals.

There has been much literature documenting the occurrence of salmonella in livestock and domestic birds, and in slaughterhouses and meat packing plants.

2.2.2.1 Poultry. Wild and domestic fowl and related products are well established as being susceptible to salmonella contamination problems. In the poultry industry in Canada, up to 1 in 4 birds on the market are contaminated with salmonella. Also, salmonella are frequently present in eggs and egg powder. They gain entrance into the eggs either as a result of ovarian infection, or by penetration of the egg shells (Clarenburg et al., 1952; Edwards, 1958).

In Florida, from a flock of domestic fowl, including chickens, turkeys, and ducks (Galton et al., 1955), 23 salmonella serotypes were isolated from internal organs (heart, liver, spleen, gall bladder), cecal contents, feces, and from skin and cloacal swabs. The contamination of meat and fish in a butcher shop in West Sussex, England (Galbraith et al., 1962), from slaughtered ducks infected with *S. saint paul*, resulted in several cases of food poisoning. In Budapest, slaughterhouse specimens of chickens, geese, turkeys, ducks, and pigeons contained salmonella (Takacs and Nagy, 1973) as did certain crows, finches, canaries, and nightingales in Holland (Clarenburg et al., 1952).

**FIGURE 2 PATHWAYS OF SALMONELLA TRANSMISSION
WITHIN A MEAT PACKING PLANT**



For "highly pollutional" poultry plant effluents at Millsboro, Delaware (Nemerow, 1967), a two-stage oxidation pond treatment system was installed to remove a major portion of the organic matter and to reduce the B.O.D. After chlorination, maximum coliform, fecal coliform, and fecal streptococcus counts/100 ml were reduced from 6.0×10^6 , 1.2×10^6 , and 2.0×10^6 , respectively, in the influent to 5.4×10^4 , 6.0×10^3 , and 6.3×10^4 , respectively, in the final effluent.

2.2.2.2 Pork. Hardy and Galton (1955) demonstrated that pork products in English open markets were frequently contaminated with salmonella. Meat processing practices were considered to be the major cause of the dissemination of these organisms resulting from both the rapid spread of infection among hogs a few hours or days before slaughter, and the wide distribution of salmonella within the abattoirs. Dehairing machines, in particular, were implicated as a source of contamination (Galton et al., 1954; Hardy and Galton, 1955).

McDonagh and Smith (1958) reported that of the common domestic animals slaughtered for human consumption, pigs were the most frequently infected with salmonella. In their particular study, 50% of the batches of mesenteric glands from apparently normal pigs contained salmonella, and the serotypes isolated, including *S. bovis* and *S. morbificans*, originated from contaminated feeds. Serotypes isolated from mesenteric glands, blood, cecal contents, and fecal swabs from freshly slaughtered pigs in Northern Ireland (Newell et al., 1959) originated, at least partly, from contaminated pig meal.

Other reports of salmonella isolations, from mesenteric lymph nodes, liver, small intestine, cecal contents, and fecal swabs of slaughtered pigs, have been made by Weissmann and Carpenter (1967) who found the incidence of infection in pig carcasses to be 56% in abattoirs in Georgia, and by Sinha et al. (1970), Ahuja and Khera (1972), Ghosh (1972), Lee et al. (1972), Carpenter et al. (1973), and McCaughey (1973). These authors observed that a considerable level of infection was common in pigs and hence in their meat products, and indicated that this was the result of a combination of: (1) infected pig meals, (2) cross contamination in transport and lairage, (3) reactivation of latent infections within the lairages brought on by stressful conditions before slaughter.

A bacteriological examination of pig slaughterhouse sewage effluent (Kucharski, 1970) revealed *E. coli* numbers of 10^8 /ml. Three salmonella serotypes, including *S. dublin*, *S. heidelberg*, and *S. newington*, were isolated. The slaughterhouse sewage proved to be a good bacterial growth medium.

2.2.2.3 Bovines. Similarly to pigs, bovines, also, may be either symptomless carriers of, or diseased with salmonella. It is claimed, however, that in meat packing, the level of the incidence of salmonella in cattle carcasses is generally lower than in pig carcasses (Weissman and Carpenter, 1967). Salmonella carriers among apparently healthy cows in slaughterhouses, discovered by isolations from mesenteric lymph nodes, fecal samples, thyroids, and livers were discovered in Italy (Izzi et al., 1971) and Germany (Habermalz, 1973)

Salmonellosis in cattle resulting in disease symptoms such as diarrhoea, mastitis, photosensitization, dehydration, and abortion have been reported (George et al., 1972; Russell, 1973). Paratyphoid, contracted by a herd of cattle in England from sewage-contaminated stream water, led to infections in the farm workers, and subsequently, in people in a village downstream (George et al., 1972).

2.2.2.4 Other animals. Other animals sent to slaughter which can be carriers of salmonella include sheep, goats, and horses. In South America, fecal material, from horses slaughtered for human consumption, contained 7 serotypes (Quevedo et al., 1973). In India, of one thousand healthy sheep tested for salmonella by means of rectal swabs (Sethi et al., 1968), close to 5% were excretors and 15 different serotypes were isolated. Also in India (Kumar et al., 1973), from feces, mesenteric lymph nodes, livers, and spleens of slaughtered sheep and goats, 22 serotypes were identified. Over 3% of these animals were carriers, and *S. typhimurium*, was the most prevalent serotype. These authors considered that these findings had significant public health implications since salmonellosis is a prevailing affliction in India.

2.3 Potential Hazards of Salmonella in Waterways

Non-disinfected wastes from, for example, municipal sewage treatment plants, meat packing plants, and livestock feedlot wastes on farms (Miner et al., 1967) are major contributors of salmonella to waterways. The release of salmonella to freshwater systems poses an immediate public health threat and serves as a principal mechanism by which to continue the circulation of

salmonella through the natural system, and to perpetuate the infection-reinfection processes of animals, and ultimately, man. Birds, rodents, and insects, which come into contact with contaminated waters, may, in turn, become salmonella carriers and disperse these organisms to other waterways, including drinking water supplies, and to animal and human foodstuffs (Clarenburg et al., 1952).

A number of studies on the presence of salmonella in sewage-polluted rivers and harbours, and in the wild fowl which inhabited these waters, revealed that serotypes found in the water were also present in gulls, terns, ducks, and other birds (Edwards, 1958). The involvement of birds in the transmission of salmonella is not a frivolous notion. A serious epidemic of *S. typhimurium* in sheep, in the state of South Australia (Watts, 1952), was spread from property to property by carnivorous birds. These birds preyed upon the carcasses of infected sheep and subsequently fouled the water supplies of other farms. They remained carriers for up to 27 days after feeding upon contaminated meat, while contaminated soils and water supplies remained infected for up to 200 days and 119 days respectively.

There is evidence that in salmonella-contaminated waters, these organisms may be present in fish, shellfish, turtles (Edwards, 1958), and other aquatic life. The occurrence of salmonella in fish is significant in view of the following observations. First, the bacterial flora of fish may reflect the bacteriological status and sanitary condition of the waters from which they originated. Fish can retain, in their digestive tracts and skin mucous, many human pathogens including *E. coli*, *Salmonella*, *Shigella*, *Staphylococcus*, and *Clostridium botulinum* (Potter and Baker, 1961). Martin (1966) recovered, from the intestines of fish caught in a river below a sewage treatment plant, 17 salmonella serotypes. The fish continued to excrete these organisms for 29 days while in holding tanks. Thus fish can be vectors of bacterial contamination to unpolluted waters and can play a role in determining the bacterial flora of their environment (Potter and Baker, 1961).

Fish may be carriers of other human pathogens, including viruses, yeasts, and fungi, as well as bacteria. These pathogens may survive and even multiply within the fish, thus threatening their use for human consumption, or for processing into fishmeal for animal feeds. As an example, in Japan,

fish are the most common food responsible for the spread of cholera (Potter and Baker, 1961).

Finally, at high enough concentrations, human pathogens may be lethal to fish (Martin, 1966), particularly since the combined effects of thermal, chemical, and bacterial pollution of the aquatic environment may lead to infections with mutants of these pathogens (Snieszko, 1964). Allen and Pelczar (1967) found that white perch, fed large numbers of *Shigella dysenteriae* or *Salmonella paratyphi* A, died, and that the same species of inoculated bacteria could be recovered from their organs.

2.3.1 Spray Irrigation with Sewage Effluents. The value of human and animal wastes for soil amendment and nutritional enrichment has long been appreciated. Workers who have studied both the benefits and health hazards of sewage disposal on land generally would agree that "as a preventative measure and good public health practice, land disposal of sewage effluents would reasonably include disinfection after secondary treatment (Durrani, 1971; Benarde, 1973)". In a review, Krishnaswami (1971) recommended that "raw waste waters should not be used for irrigation of any type, anywhere," and that effluents to be used for this purpose should be treated and always disinfected effectively.

The major problems associated with the use of improperly treated waste water for irrigation are: (1) the occupational hazard to persons working in contact with the waste, (2) disease transmission to animals grazing on sewage irrigated land, (3) dangers associated with human consumption of ground and surface waters with pathogenic organisms.

Instances where cattle have become infected with salmonella, after grazing on pasture which had been irrigated with manure slurry from feedlot wastes, have been reported by Miner et al. (1967), Jack and Hepper (1969), Krishnaswami (1971), Haig (1972), Taylor (1973), and Jones and Matthews (1975). In a thorough study on the "Health Aspects of Sewage Effluent Irrigation", sponsored by the government of British Columbia, Parsons et al. (1975) have reviewed 63 human disease outbreaks associated with the irrigation of soils and crops with untreated or poorly treated sewage, in various countries throughout the world.

Of considerable importance, if non-disinfected wastes are to be disposed on agricultural soils, is the survival time of pathogens within the effluents. Although these pathogens are comprised of viruses and parasites, as well as several bacteria, the following discussion will be restricted to salmonella.

The survival of salmonella in soils depends on the following:

(1) the composite effects of the microbial flora, (2) amount of organic matter and clay, (3) pH, (4) chemical toxicity, (5) available nutrients for persistence and regrowth, (6) temperature, (7) moisture, and (8) grass cover for protection from the sun. The duration of survival of salmonella in soil has been reported to range from 24 hour in exposed, dry soils, to as long as 2 years in frozen, moist soils (Geldreich, 1973). Salmonella survival in soil and water, for several days and even weeks, has been recorded frequently (Gallagher and Spino, 1968; Hendricks, 1971; Krishnaswami, 1971; Haig, 1972; Benarde, 1973).

2.3.2 Drug Resistance Transfer in the Aquatic Environment. In 1959, Japanese scientists first postulated the transfer of chemotherapeutic agent resistance, from one bacterium to another, carried by R factors. R factors are extra chromosomal pieces of nucleic acid, also known as episomes, which can be transferred between gram negative organisms by means of conjugation or transduction. Details of the specific mechanisms involved have been outlined by Watanabe (1964), Anderson (1968), and Baldwin (1970). Large numbers of drug resistant organisms have evolved as a consequence of widespread use of antibiotics in medicine and agriculture, and through subsequent transfers of the resistance factors to other organisms, both *in vitro* and *in vivo*.

Consistent use of antibiotics in agriculture has been made in livestock feeds and drinking water, both to promote growth and to prevent disease (Kiser et al., 1971). As a result, domestic animals now show a much greater prevalence of multi-resistant organisms with R factors as compared to wild animals (Huber et al., 1971). Also, resistant *E. coli* no longer occur predominantly in animals which are fed antibiotics continually, but are present in animals only occasionally exposed to antibiotic treatment.

The upsurge of resistant pathogenic and non-pathogenic alimentary tract bacteria now encompasses virtually all members of the Enterobacteriaceae, including *E. coli*, *Klebsiella*, *Citrobacter*, *Shigella*, *Aerobacter*, *Salmonella*, *Proteus*, and *Providencia*, along with other organisms such as *Staphylococcus* and *Pseudomonas* (Anderson, 1968; Sturtevant and Feary, 1969; and Huber et al., 1971). These may be singly or multiply resistant to such antibiotics as streptomycin, tetracycline, chloramphenicol, kanamycin, ampicillin, furazolidone (Guinee, 1971), erythromycin, cephaloridine (Kiser et al., 1971), oxytetracycline, neomycin, and sodium nalidixate, and the sulfonamides (Williams Smith, 1970).

The public health and animal health significance of this increase in drug resistant organisms in the environment is several fold. First, antibiotic resistant bacteria may be transferred from animals to man, through either direct contact or contaminated food, leading to wide spread untreatable diseases in man (Kiser et al., 1971). The transmittal from animals to man of *S. typhimurium*, carrying transferable drug resistance, was implicated where there were 6 human deaths (Anderson, 1968).

Secondly, there can be *in vivo* transfer of drug resistance from non-pathogenic to pathogenic intestinal tract organisms, in either humans or animals, resulting in infections and diseases which are difficult to treat. In England, a bovine outbreak of *S. typhimurium* was reported in which the *E. coli* and infecting *S. typhimurium* had identical resistance patterns (Anderson, 1968).

Finally, there may be ready transfer of drug resistance between susceptible pathogenic and non-pathogenic gram negative organisms in the environment, possibly leading to resistant infections or carrier states in birds, fish, animals, and humans through contact with contaminated water, food, etc. In the environment in Great Britain, many multi-resistant *Salmonella* were isolated from meat-producing areas. These salmonella originated principally from pigs, turkeys, and chickens. However, infected cows, horses, and sheep contributed as well (Kiser et al., (1971).

Resistant coliforms and fecal coliforms have been isolated, in

Britain and the United States, from raw and treated sewage, from rivers in proximity to urban areas, and from farm feedlot runoff (Williams Smith, 1970; Kiser et al., 1971; Sturtevant and Feary, 1969; Sturtevant et al., 1971; Feary et al., 1972; Koditschek and Guyre, 1974). At least 1% of the lactose-fermenting enteric organisms recovered from raw sewage were multi-resistant, and this percentage, after sewage treatment, did not decrease.

In view of the above, and from evidence of drug resistance transfer from *E. coli* to *Salmonella* serotypes (Anderson, 1968; Kiser et al., 1971; Feary et al., 1972; Koditschek and Guyre, 1974), it should be evident that the disinfection of municipal sewage and meat packing plant wastes would result in a decrease in the release of enteric organisms, possibly carrying R factors, and would be a step in the control of drug resistance transfer.

3.0 METHODS

3.1 Sampling Procedures

Samples were collected in sterile polypropylene one litre bottles containing sodium thiosulphate to give a final concentration of 100 mg per litre (A.P.H.A. Standard Methods, 1972).

Modified Moore swabs (Spino, 1966) were hung overnight in the streams to be investigated. The swabs were constructed on a 12" x 12" mesh of chicken wire. Five 1.5" x 9" gauze strips, each made up of 6 layers of gauze, were gathered together at one end and tied with string to the wire mesh. The other ends of the strips were spread out on the frame and separately tied, in order to expose the maximum surface area to the water.

3.2 Bacteriological Procedures

All samples were subjected to A.P.H.A. Standard Methods (1972) membrane filtration procedures for the estimation of coliform, fecal coliform, and fecal streptococcus densities. For these tests, Millipore 0.45 u, white, gridded, membrane filters (H.A.W.G. 047) were used. A.P.H.A. (1972) methods were used also for the 35°C and 20°C standard plate counts.

Enrichment media, and solid and broth media were Bacto Brand, supplied by Difco Laboratories, Detroit, Michigan, unless otherwise specified.

3.2.1 Coliform Density Determinations. The appearance of reddish colonies with a green-gold surface sheen, after incubation at $35 \pm 0.5^{\circ}\text{C}$ for 24 ± 2 hour, on m-Endo Agar L.E.S., was interpreted as evidence of the presence of coliforms. Colony counts were calculated to establish the number of coliforms per 100 ml water.

3.2.2 Fecal Coliform Densities. Blue or blue-grey coloured colonies, on m-F.C. Agar with rolsolic acid, were considered typical of fecal coliform colonies. Incubation of the plates, sealed in Whirl Pak bags, was at $44.5 \pm 0.2^{\circ}\text{C}$ in a circulating water bath for 24 ± 2 hour. Counts were recorded as fecal coliforms per 100 ml sample.

3.2.3 Fecal Streptococcus Density Determinations. Fecal streptococcus colonies were pink or maroon coloured on m-Enterococcus Agar. Incubation was at $35 \pm 0.5^{\circ}\text{C}$ for 48 ± 4 hour, and counts were recorded as fecal streptococci per 100 ml sample.

3.2.4 Salmonella Culturing Methods. Three of the five strips from the Moore swab were each transferred to 500 ml of Tetrathionate Broth which had been prewarmed to 41.5°C . The remaining two strips were each placed in 500 ml of prewarmed Selenite Broth. From the samples collected in the one litre polypropylene bottles, 100 ml and 10 ml aliquots were added to 500 ml prewarmed Tetrathionate and Selenite Broths. These enrichment broths were incubated at $41.5 \pm 0.2^{\circ}\text{C}$ for 20 ± 2 hour.

Subsequently one X.L.D. Agar and one Brilliant Green Agar plate were streaked with a loopful of broth taken from the surface. After 20 ± 2 hour incubation at 41.5°C , colonies typical for *Salmonella* (shiny black colonies on X.L.D. Agar and pink colonies on Brilliant Green Agar), were streaked on Phenylalanine Agar and MacConkey Agar and incubated overnight at $35 \pm 0.5^{\circ}\text{C}$. Lactose and phenylalanine deaminase negative colonies were inoculated into tubes to test for motility, and the lack of indole production, citrate and malonate utilization, and urease activity. T.S.I. Agar slants were used to determine the production of acid, gas, and H_2S .

Cultures with characteristics typical for the genus *Salmonella* were further identified according to the methods of Edwards and Ewing (1972).

Somatic (0) antigens were tested for by means of slide agglutinations with polyvalent 0 antisera and the 0 specifics. Spicer Edwards pooled H antisera were used for the flagellar antigens. Organisms confirming to this stage were subcultured and representative cultures of each serotype were sent to the National Enteric Reference Centre, Department of National Health and Welfare, Ottawa, for confirmation of the identifications.

3.2.5 Coliform and Fecal Coliform Identifications. The methods of Edwards and Ewing (1972) and Cowan and Steel (1965) were implemented to generically identify colonies which appeared, on their respective isolation media, to be typical coliforms and fecal coliforms. The following biochemical tests were performed on the isolates: Catalase, Cytochrome Oxidase, Glucose O.F., Glucose, Lactose, Indole, Methyl Red, Vogues Proskauer, Simmon's Citrate, H₂S (on T.S.I. Agar), Ornithine Decarboxylase, Motility, E.C. and Gelatin. The Cytochrome Oxidase test was included in the identification scheme in order to differentiate quickly *Aseomonas* from the Enterobacteriaceae.

3.2.6 Antibiotic Resistance of Coliform, Fecal Coliform, and Salmonella Isolates. Colonies of the coliform, fecal coliform, and salmonella isolates to be tested were inoculated into tubes of nutrient broth and incubated at 37°C for about 4 hours. Portions of each broth were poured onto plates of Sensitest Agar (Oxoid), swirled to wet the entire agar surface, and then the excess was discarded. Aseptically, Multodisk (Oxoid) antibiotic sensitivity testing disks were placed on the plates, and these were incubated overnight at 37°C. Antibiotics employed were Chloramphenicol, 10 ug, Erythromycin, 10 ug, Novobiocin, 5 ug, Cloxacillin, 5 ug, Penicillin G, 1.5 units, Ampicillin, 2 ug, Streptomycin, 10 ug, and Tetracycline, 10 ug. Diameters of the zones of growth inhibition around the disks were measured to determine the antibiotic sensitivities of the test organisms.

3.2.7 Salmonella Survival in Water. Salmonella survival experiments were conducted in conjunction with tests with Rainbow Trout fingerlings and salmonella at the Bioassay Laboratory, E.P.S. Edmonton. 1,400 ml of nutrient broth, containing a stationary phase culture of *S. typhimurium*, were added to 20 l of water to bring the final concentration of cells to 7.0×10^6 /ml. Similarly, stationary phase cells, which had been washed in phosphate buffer,

were added to 20 ℓ of water to give a concentration of cells of 5.0×10^6 /ml. The water was Edmonton City water which had been sand filtered to remove solids, sterilized by means of U.V. lights, carbon filtered, and the pH adjusted from 9.3 to 7.5 by the addition of CO_2 . During the experiment, the water was aerated and maintained at 15°C .

Over a period of 13 days, samples were removed from the two experimental tanks, 10 ml aliquots were serially diluted in 90 ml phosphate buffer dilution blanks, and 0.1 ml portions of the appropriate dilutions were spread on X.L.D. Agar plates. Shiny black colonies were counted, after a 24 hour incubation period at 35°C , in order to obtain the count of salmonella cells per ml.

3.2.8 Fish as Potential Carriers of Pathogens. Five rainbow trout fingerlings were placed in each of a control tank and 5 tanks containing 20 ℓ of water and approximately 10^6 /ml cells of *S. typhimurium* as described in section 3.2.7. The water was aerated and maintained at 15°C .

At intervals, during a two week period, fish were sacrificed and scrapes of the skin mucous and intestinal content samples were streaked on X.L.D. agar and incubated at 35°C for 24 hour to test for the production of shiny, black colonies, typical for salmonella.

In an additional experiment, while 2 fish from one of the above tanks were tested immediately after removal for salmonella, the remaining 3 fish were placed in 20 ℓ of fresh water. After 3 days, skin scrapes and the intestinal contents of these fish were streaked on X.L.D. agar to determine if they were still salmonella carriers.

4.0 RESULTS

4.1 Results from Individual Meat Packing Plants

4.1.1 Alberta Red Meat Slaughterhouses and Packing Plants.

4.1.1.1 Edmonton 1. This plant slaughters and processes 450 cattle and 1,000 hogs per day and waste discharge to the municipality totals about 750,000 imperial gallons per day. Waste treatment, considered primary, is accomplished in settling/flotation tanks with fat skimmers. Settled solids and skimmed fat from the tanks are trucked away for land disposal. Influent to the treatment tanks arrive from a fat line from rendering processes, and

from a blood and manure line.

Samples were obtained from the influent lines and from the final effluent from the settling/flotation tanks, As illustrated in Table 1, indicator organism numbers were comparable to those usually found in raw sewage. It is interesting to note that in January, the numbers of indicator bacteria were several times greater in the final effluent than in the influent.

Salmonella (Table 2) were isolated from as little as 10 ml of both influent and effluent samples. A total of 13 different serotypes were isolated and identified.

4.1.1.2 Edmonton 2. This plant slaughters and processes 400 cattle and 2,500 hogs per day and effluent discharge to the municipal lagoons amounts to 800,000 imperial gallons per day. Wastes conducted to the settling tanks consist of those from the residues of rendering edible fat trimmings, and from inedible wastes, manure, and wash water. Treatment processes of these are primary settling, skimming, and chemical treatment called pacific separation.

Samples of the influent wastes from edible product rendering, of inedible wastes, and of final effluent were taken. Bacterial indicator organism numbers, in both the influents and effluents (Table 3), were comparable to those usually demonstrated in raw sewage. Salmonella were isolated from as little as 10 ml of final effluent, and 4 serotypes, including *S. tennessee*, *S. californica*, *S. bredeney*, and *S. schwarzengrund*, were identified.

4.1.1.3 Edmonton 3. This plant slaughters and processes 200 to 300 cattle and 500 to 1,000 hogs per day, and consumes approximately 700,000 imperial gallons of water. Wastes, consisting of kill floor wash water and cooling water, are directed to a grease skimmer then to a settling tank. Manure is not incorporated with these wastes, but instead, is trucked away.

On the actual sampling dates, the settling tank was not in operation and samples taken from the skimmer comprised, therefore, the final effluent. Numbers of indicator bacteria obtained are listed in Table 4. Salmonella were isolated from as little as 10 ml of sample and 3 different serotypes,

TABLE 1. RED MEAT PACKERS, EDMONTON 1. INDICATOR ORGANISMS

Date	Sample Location	MF COUNT PER 100 ML		Fecal Coliform	Fecal Strep.	S.P.C. Per ML	
		Coliform	Coliform			35°C	20°C
8-10-74	Fat tank influent	15,000,000	1,100,000	200,000	12,000,000	810,000	
	Blood & manure influent	45,000,000	2,400,000	250,000	17,000,000	6,300,000	
	Final effluent	27,000,000	640,000	140,000	25,000,000	15,000,000	
6-1-75	Combined Flotation tank influent	5,700,000	2,900,000	220,000	650,000	350,000	
7-1-75	Combined Flotation tank influent	2,800,000	2,200,000	100,000	430,000	170,000	
6-1-75	Final effluent	26,000,000	3,000,000	290,000	6,600,000	5,000,000	
7-1-75	Final effluent	43,000,000	1,460,000	470,000	5,300,000	7,500,000	

TABLE 2. RED MEAT PACKERS, EDMONTON 1. SALMONELLA SEROTYPES ISOLATED FROM:

Date	Sample Location	Moore Swab	100 ml	10 ml
8-10-74	Fat tank influent	S. kentucky	Not done	S. newington
		S. typhimurium var. copenhagen		S. kentucky
	Blood & Manure influent	S. montevideo		
		S. typhimurium	Not done	S. typhimurium var. copenhagen
				S. newington S. derby S. halmstad S. typhimurium
6-1-75	Final effluent	S. anatum	Not done	S. newington
		S. montevideo		S. kentucky
		S. kentucky		
		S. newington		
		S. typhimurium var. copenhagen		
		S. bredeney		
		S. infantis		
		S. oranienburg		
		S. senftenberg	S. halmstad	S. kentucky
		S. halmstad		S. thompson
		S. newington		
		S. kentucky		

TABLE 3. RED MEAT PACKERS, EDMONTON 2. INDICATOR ORGANISMS AND SALMONELLA SEROTYPES

Date	Sample Location	MF COUNT PER 100 ML			Fecal Strep.	S.P.C. Per ML	
		Coliform	Coliform	Coliform		35°C	20°C
6-1-75	Edible products	3,500,000	1,000,000	3,700,000	380,000	380,000	380,000
7-1-75	Edible products	92,000,000	4,600,000	860,000	3,400,000	2,800,000	2,800,000
6-1-75	Inedible wastes influent	23,000,000	2,500,000	2,600,000	6,600,000	6,400,000	6,400,000
7-1-75	Inedible wastes influent	15,000,000	160,000	240,000	670,000	870,000	870,000
6-1-75	Final effluent	13,000,000	4,500,000	2,000,000	3,500,000	2,200,000	2,200,000
7-1-75	Final effluent	32,000,000	4,300,000	550,000	4,700,000	5,700,000	5,700,000
3-2-75	Final effluent	10,000,000	2,000,000	2,100,000	2,100,000	1,300,000	1,300,000

SALMONELLA SEROTYPES ISOLATED FROM:				
Date	Sample Location	Moore	Swab	10 ml
6-1-75	Final effluent	S. tennessee	S. tennessee	S. tennessee
		S. schwarzengrund	S. californica	S. californica
3-2-75	Final effluent	Not done	Negative	S. bredeney
				Negative

TABLE 4. RED MEAT PACKERS, EDMONTON 3. INDICATOR ORGANISMS AND SALMONELLA SEROTYPES

Date	Sample Location	MF COUNT PER 100 ML				S.P.C. Per ML	
		Coliform	Fecal Coliform	Fecal Strep.	35°C	20°C	
6-1-75	Final effluent	28,000,000	3,500,000	3,300,000	1,100,000	1,300,000	
7-1-75	Final effluent	36,000,000	3,100,000	490,000	9,100,000	9,800,000	
SALMONELLA SEROTYPES ISOLATED FROM:							
Date	Location	Moore Swab		100 ml		10 ml	
		Negative	S. montevideo	S. infantis	S. senftenberg		
6-1-75	Final effluent						

S. montevideo, *S. infantis*, and *S. senftenberg*, were identified.

4.1.1.4 Edmonton 4. This plant slaughters 70 to 75 horses per day. There is primary settling of the waste which is comprised of slaughterhouse waste, including manure, and wash water. About 600,000 imperial gallons of water per day are consumed.

Indicator organism numbers in the final effluent (Table 5) were somewhat lower than those found in the final effluents of the beef and pork processing plants. This may have been a dilution effect as a result of the large amount of water employed.

During sampling proceedings in this plant, the Moore swab was lost. However, 3 different salmonella serotypes were isolated from 100, 10, and 1 ml samples, and in three instances, *S. good* was identified. This serotype is rarely found in Canada (Laidley, personal communication), but is more commonly found in horses and horse slaughterhouses in South America, particularly in Argentina and Peru.

4.1.1.5 Red Deer 5. Approximately 200 cattle and 600 hogs are slaughtered and processed per day in this plant, producing 250,000 imperial gallons of waste water per day. Primary treatment consists of air flotation and settling, and the total waste is comprised of that from the kill floor, and from the rendering of edible and inedible products. Final effluent samples, only, were taken.

Indicator bacteria numbers (Table 6) were lower on Monday, January 20, than on the following day. A possible explanation for this occurrence is that there may be a gradual build-up of waste material, and hence bacteria, as the week progresses, after the weekend shutdown. The Moore swab was lost in this study. However, *S. newington* was isolated from 100 and 10 ml effluent samples.

4.1.1.6 Red Deer 6. This plant slaughters about 350 cattle per day producing 180,000 imperial gallons of waste water per day. Treatment consists of primary settling and fat skimming.

Final effluent samples were examined for indicator bacteria and for salmonella (Table 7). The negative results from the attempts to isolate

TABLE 5. RED MEAT PACKERS, EDMONTON 4. INDICATOR ORGANISMS AND SALMONELLA SEROTYPES

Date	Sample Location	MF COUNT PER 100 ML				S.P.C. Per ML	
		Coliform	Fecal Coliform	Fecal Strep.	35°C	20°C	
14-1-75	Final effluent	12,000,000	1,300,000	510,000	58,000	140,000	
14-1-75	Final effluent	7,400,000	1,500,000	680,000	380,000	330,000	
SALMONELLA SEROTYPES ISOLATED FROM:							
Date	Sample Location	Moore Swab			10 ml		
		Lost			S. good		
14-1-75	Final effluent				S. good		
					S. java		

TABLE 6. RED MEAT PACKERS, RED DEER 5. INDICATOR ORGANISMS AND SALMONELLA SEROTYPES

Date	Sample Location	MF COUNT PER 100 ML				S.P.C. Per ML	
		Coliform	Fecal Coliform	Fecal Strep.	35°C	20°C	
20-1-75	Final effluent	6,500,000	1,100,000	380,000	100,000	50,000	
21-1-75	Final effluent	53,000,000	5,100,000	2,700,000	1,300,000	1,700,000	
SALMONELLA SEROTYPES ISOLATED FROM:							
Date	Sample Location	Moore Swab		100 ml		10 ml	
		Lost		S. newington		S. newington	
21-1-75	Final effluent						

TABLE 7. RED MEAT PACKERS, RED DEER 6. INDICATOR ORGANISMS AND SALMONELLA SEROTYPES

Date	Sample Location	MF COUNT PER 100 ML				S.P.C. Per ML	
		Coliform	Fecal Coliform	Fecal Strep.	35°C	20°C	
20-1-75	Final effluent	2,600,000	1,600,000	450,000	140,000	24,000	
21-1-75	Final effluent	2,400,000	740,000	1,400,000	180,000	210,000	
SALMONELLA SEROTYPES ISOLATED FROM:							
Date	Sample Location	Moore Swab		100 ml	10 ml		
20-1-75	Final effluent	Negative	Negative	Negative	Negative		

salmonella may have been due to inadequate methodology. It is possible, however, that the cattle being slaughtered at the time of sampling were salmonella-free. Incidents of only sporadic isolations of salmonella from slaughterhouses are well documented.

4.1.1.7 Red Deer 7. This plant slaughters 500 hogs per day, and produces approximately 450,000 imperial gallons of waste water. There is no waste treatment. Samples of the kill floor effluent and final effluent were obtained. The indicator bacteria numbers (Table 8) in the final effluent were significantly greater on Tuesday, January 21, than found the previous day. A similar situation was observed in the plant designated Red Deer 5 (Section 4.1.1.5), and again, an explanation may be that waste material, and hence the number of bacteria, accumulates as the week progresses, after the weekend shut down. Moore swabs, placed in the kill floor and final effluent streams, were lost. The 100 and 10 ml effluent samples proved negative for the presence of salmonella.

4.1.1.8 Calgary 8.

This plant slaughters and processes 250 to 300 cattle per day and processes, but does not slaughter, pork. The 900,000 imperial gallons of waste water undergoes primary settling only, and retention time in the settling tank is about 3 hours. The influent and effluent of the tank were screened for indicator bacteria, and the final effluent only, was tested for the presence of salmonella. There was no reduction in the numbers of indicator organisms after settling (Table 9), and attempts to isolate salmonella failed, even from the Moore swab.

4.1.1.9 Calgary 9. This plant slaughters and processes 485 cattle, 850 hogs, and 100 sheep per day. The 750,000 imperial gallons of waste water produced daily are treated by what is considered to be the "best practical treatment" for this type of waste. The scheme of the treatment facilities at this Calgary plant is shown in Figure 3. Wastes from the rendering of edible and inedible products and from the kill floor are directed, through a fat line, to a primary clarifier (flotation and settling) fat tank. Other wastes, principally manure, are collected in a manure settling tank. Settled solids from these tanks are screened and recirculated back to the tanks. The

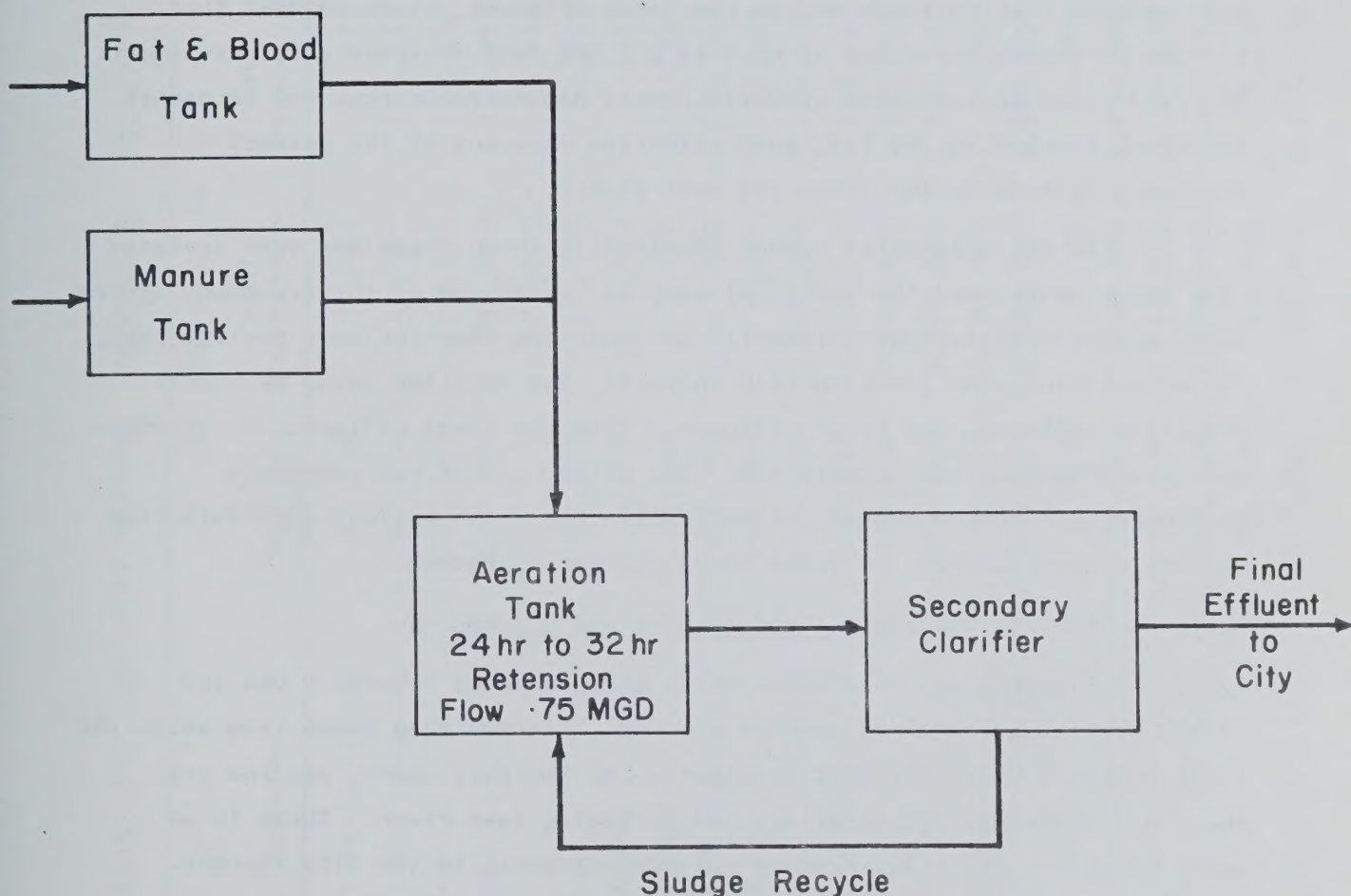
TABLE 8. RED MEAT PACKERS, RED DEER 7. INDICATOR ORGANISMS AND SALMONELLA SEROTYPES

Date	Sample Location	MF COUNT PER 100 ML				S.P.C. Per ML	
		Coliform	Fecal Coliform	Fecal Strep.	35°C	8°C	20°C
20/1/75	Kill floor	5,000,000	13,000	30,000	190,000	27,000	
21/1/75	Kill floor	22,000,000	1,300,000	2,900,000	1,000,000	1,600,000	
20/1/75	Final effluent	290,000	120,000	38,000	5,200	110,000	
21/1/75	Final effluent	190,000,000	12,000,000	TNTC (T)	1,100,000	880,000	
SALMONELLA SEROTYPES ISOLATED FROM:							
Date	Sample Location	Moore Swab		100 ml	10 ml		
21/1/75	Kill floor	Lost	Negative	Negative	Negative		
21/1/75	Final effluent	Lost	Negative	Negative	Negative		

TABLE 9. RED MEAT PACKERS, CALGARY 3. INDICATOR ORGANISMS AND SALMONELLA SEROTYPES

Date	Sample Location	MF COUNT PER 100 ML			S.P.C. Per ML	
		Coliform	Coliform	Fecal Strep.	35°C	20°C
11/3/75	Final influent	21,000,000	1,600,000	380,000	5,800,000	14,000,000
11/3/75	Final effluent	22,000,000	600,000	570,000	12,000,000	4,300,000
SALMONELLA SEROTYPES ISOLATED FROM:						
Date	Sample Location	Moore Swab				
					100 ml	10 ml
11/3/75	Final effluent	Negative			Negative	Negative

**FIGURE 3 SCHEME OF WASTE TREATMENT FACILITIES,
RED MEAT PACKERS, CALGARY 9**



effluents from these primary clarifiers are combined and pumped to an extended aeration tank, where 600 gallons per minute are processed. Material in the aeration tank is circulated and aerated by rotors, and retention time is 24 to 32 hour. Effluent from the aeration tank goes to a secondary clarifier and sludge from this clarifier is pumped back to the aeration tank, while the remaining effluent is received by the municipal sewage system.

From data on the numbers of indicator organisms (Table 10) in the aeration tank influent and in the final effluent, it is evident that in some instances there was as much as a 1,000 fold decrease after treatment. The reductions in indicator bacteria counts demonstrated that the secondary treatment process is, by far, more effective than any of the primary treatment systems in the other red meat plants.

In the salmonella survey (Table 11), these organisms were isolated from Moore swabs, and 100 and 10 ml samples. Sections of the treatment system which proved positive for salmonella at least one time included the fat tank, the manure tank, the aeration tank influent, the aeration tank, secondary clarifier influent, and final effluent. From the final effluent, *S. alachua* and *S. senftenberg* were identified. The efficiency of the secondary treatment in reducing numbers of salmonella cannot be estimated at this time because a quantitative, in depth study was not performed.

4.1.2 Alberta Poultry Slaughterhouses and Processors.

4.1.2.1 Edmonton 1. This plant kills and processes between 9,000 and 10,000 broilers, fowl, and turkeys per day. The two main areas from which the final combined waste effluent originates are the kill floor, and the area where defeathering, eviscerating, and packaging take place. There is no waste treatment and effluents are released directly to the city lagoons.

Indicator bacterial numbers in the final effluent (Table 12) fluctuated but generally were comparable to those obtained from raw sewage. Three different serotypes of salmonella were isolated from the Moore swab, and 100 and 10 ml samples of the effluent. These were *S. kentucky*, *S. californica*, and *S. thompson*.

TABLE 10. RED MEAT PACKERS, CALGARY 9. INDICATOR ORGANISMS

Date	Sample Location	MF COUNT PER 100 ML				S.P.C. Per ML	
		Coliform	Fecal Coliform	Fecal Strep.	35°C	20°C	
29-10-74	Aeration Tank Influent	430,000	320,000	94,000	3,100,000	2,900,000	
30-10-74	Aeration Tank Influent	1,300,000	340,000	220,000	1,300,000	970,000	
29-10-74	Manure Tank	1,800,000	760,000	290,000	1,500,000	610,000	
30-10-74	Manure Tank	800,000	390,000	350,000	630,000	440,000	
29-10-74	Fat Tank	*	23,000	13,000	36,000	48,000	
30-10-74	Fat Tank	75,000	17,000	57,000	85,000	100,000	
29-10-74	Final Effluent	3,200	1,600	300	17,000	8,400	
30-10-74	Final Effluent	7,500	700	200	17,000	15,000	
11-2-75	Aeration Tank Influent	18,000,000	2,500,000	580,000	6,300,000	4,000,000	
11-2-75	Final Effluent	100,000	9,000	12,000	53,000	310,000	
19-2-75	Aeration Tank Influent	27,000,000	760,000	3,200,000	8,500,000	310,000	
	Clarifier Influent	1,000,000	510,000	330,000	950,000	1,100,000	
	Final Effluent	770,000	180,000	180,000	140,000	160,000	
4-3-75	Aeration Tank Influent	30,000,000	2,700,000	1,600,000	9,000,000	1,600,000	
	Final Effluent	50,000	25,000	16,000	1,300,000	170,000	
5-3-75	Aeration Tank Influent	53,000,000	3,000,000	2,800,000	12,000,000	10,000,000	
	Final Effluent	10,000	8,000	4,000	320,000	100,000	
6-3-75	Aeration Tank Influent	60,000,000	950,000	1,200,000	5,400,000	1,400,000	
	Final Effluent	33,000	15,000	2,300	C O N T A M I N A T E D		
7-3-75	Aeration Tank Influent	15,000,000	3,600,000	600,000	6,500,000	1,600,000	
	Final Effluent	31,000	13,000	7,700	C O N T A M I N A T E D		
8-3-75	Aeration Tank Influent	73,000,000	500,000	4,500,000	1,700,000	1,900,000	

TABLE 10. Continued

8-3-75	Final Effluent	37,000	5,000	2,400	C O N T A M I N A T E D
11-3-75	Aeration Tank Influent	50,000,000	2,300,000	1,000,000	2,100,000 130,000
	Final Effluent	58,000	20,000	4,000	CONTAMINATED 70,000

TABLE 11. RED MEAT PACKERS, CALGARY 9. SALMONELLA SEROTYPES ISOLATED FROM:

Date	Sample Location	Moore Swab	100 ml	10 ml
30-10-74	Aeration tank	S. alachua	Negative	Negative
	Fat tank	S. sub genus I.		
	Manure tank	S. alachua	Negative	Negative
	Final effluent	Negative	Negative	Negative
11-2-75	Aeration tank influent	Sub genus I	S. bredeney	S. bredeney
	Final effluent	S. bredeney		
		S. alachua	Negative	Negative
		S. bredeney		
19-2-75	Clarifier influent	Not done	Negative	Negative
	Aeration tank influent	Negative	Negative	Negative
	Final effluent	Negative	S. senftenberg	Negative
	Aeration tank influent	S. derby	Negative	Negative
4-3-75	Final effluent	Negative	Negative	Negative
	Aeration tank influent	Not done	Negative	Negative
	Final effluent	Negative	Negative	Negative
	Aeration tank influent	Negative	Negative	Negative
5 -3-75	Final effluent	Negative	Negative	Negative
	Aeration tank influent	Negative	Negative	Negative
	Final effluent	Negative	Negative	Negative
	Aeration tank influent	Negative	S. montevideo	S. kentucky
6-3-75	Final effluent	Negative	S. kentucky	S. derby
	Final effluent	Negative	Negative	Negative
	Final effluent	S. senftenberg	Negative	Negative
	Final effluent			

TABLE 12. POULTRY PROCESSORS, EDMONTON 1. INDICATOR ORGANISMS AND SALMONELLA SEROTYPES

Date	Sample Location	MF COUNT PER 100 ML				S.P.C. Per ML	
		Coliform	Fecal Coliform	Fecal Strep.	35°C	20°C	
13-1-75	Final effluent	11,000,000	5,700,000	300,000	100,000	100,000	
14-1-75	Final effluent	38,000,000	13,000,000	310,000	100,000	170,000	
24-2-75	Final effluent	1,700,000	1,400,000	95,000	47,000	40,000	
25-2-75	Final effluent	250,000	140,000	20,000	9,100	17,000	
27-2-75	Final effluent	3,800,000	2,200,000	300,000	190,000	300,000	
SALMONELLA SEROTYPES ISOLATED FROM:							
Date	Sample Location	Moore Swab		100 ml	10 ml		
13-1-75	Final effluent	S. kentucky	Negative	Negative	Negative		
24-2-75	Final effluent	S. thompson	Negative	S. californica	S. californica		

4.1.2.2 Edmonton 2. This plant slaughters and processes 20,000 to 24,000 broilers, fowl, and turkeys daily.

On the first two sampling days, the plant owner, who was in fear that his plant was about to be prosecuted, detained the samplers for several minutes before permitting them to enter the premises. Final effluent samples obtained at these times contained extremely low numbers of indicator bacteria (Table 13). The 100 and 10 ml aliquots proved negative for the presence of salmonella, and the Moore swab, placed on the first day, was lost. On the third sampling date (February 27) advance notice was not given to the owner. Final effluent indicator organism numbers, this time, were comparable to those obtained from other meat and poultry processing industries which have no treatment or primary treatment only.

5.0 GENERAL RESULTS AND DISCUSSION

A summary of the numbers of indicator bacteria, in the influents to the treatment facilities of the red meat packing plants investigated, is shown in Table 14. These compare to those numbers usually associated with raw sewage. In sewage, coliform M.F. counts per 100 ml usually range from 10^9 to 10^6 , with the fecal coliform and fecal streptococcus counts generally between 10% and 20% less than those for the coliforms.

Fresh, liquified feces from birds and animals frequently display coliform counts of 10^5 to 10^7 , fecal coliform counts of 10^5 to 10^7 , and fecal streptococcus counts of 10^4 to 10^6 per gram. Both the 35°C and 20°C standard plate counts range from 10^5 to 10^8 per gram fresh feces (Toxopeus et al., 1974). These authors discovered that in the immediate post excretion period there was a marked increase, often greater than one order of magnitude, in the numbers of indicator bacteria, and that at this time, the FC:FS ratio was usually greater than 4. It was only after extended storage that there occurred a reversion of the FC:FS ratio to less than 0.7, the level suggested by Geldreich and Kenner (1969) as characteristic of animal waste.

In the present study, the median, geometric mean, and arithmetic mean of the FC:FS ratios, in the influents to the treatment facilities, were

TABLE 13. POULTRY PROCESSORS, EDMONTON 2. INDICATOR ORGANISMS AND SALMONELLA SEROTYPES

Date	Sample Location	MF COUNT PER 100 ML			S.P.C. Per ML	
		Coliform	Fecal Coliform	Fecal Strep.	35°C	20°C
24-2-75	Final effluent	10,000	1,000	1,000	100	12,000
25-2-75	Final effluent	700	700	100	20	110
27-2-75	Final effluent	2,100,000	940,000	220,000	51,000	80,000
SALMONELLA SEROTYPES ISOLATED FROM:						
Date	Sample Location	Moore Swab			10 ml	
		Lost			Negative	Negative
25-2-75	Final effluent					

TABLE 14. DATA SUMMARY FOR INFLUENTS TO TREATMENT FACILITIES OF RED MEAT PACKERS

	MF COUNT PER 100 ML				S.P.C. Per ML	
	Coliform	Fecal Coliform	Fecal Strep.	Ratio FC:FS	35°C	20°C
Median value	22,000,000	2,000,000	590,000	2.00	4,400,000	1,200,000
Geometric mean	19,000,000	1,200,000	500,000	2.06	2,800,000	1,100,000
Arithmetic mean	31,000,000	2,100,000	1,200,000	4.89	4,800,000	2,800,000
Minimum value	1,300,000	13,000	30,000	0.11	190,000	27,000
Maximum value	92,000,000	8,600,000	4,500,000	22.00	12,000,000	14,000,000

* values based on 18 sets of data.

2.00, 2.06, and 4.89 respectively. This is typical, according to Toxopeus et al. (1974), of very fresh animal waste.

5.1 Effects of Treatment Processes on Red Meat Packing Plant Wastes

5.1.1 Primary Treatment. Primary treatment of waste water involves the sedimentation and removal of a portion of the suspended organic and inorganic solids. As a result of this treatment, the B.O.D. and suspended solids content of waste water may be reduced by 25% to 40% and 35% to 60%, respectively (Parsons et al., 1975).

With primary treatment, there is not necessarily a reduction in numbers of bacteria and this was well demonstrated in the present study. A comparison of the orders of magnitude of the indicator bacteria counts in the influents with those in the effluents (Tables 14 and 15) from the red meat packing plants revealed that there were no reductions in these after primary treatment.

5.1.2 Secondary Treatment. Aeration tank, activated sludge secondary treatment involves the breakdown of suspended and dissolved organics by microorganisms, principally bacteria, under aerobic conditions. With this system, B.O.D. and suspended solids removals of 85% to 90% can be expected, along with 90% reductions in numbers of coliform and pathogenic bacteria (Parsons et al., 1975).

The numbers of indicator organisms in the effluents, after secondary treatment, in the Calgary plant facilities (Table 16) suggested that the system was operating efficiently. In comparing the data summaries of the influents to those of the final effluents after extended treatment (Tables 14 and 16), it was observed that, for example, the geometric means of the coliform counts decreased by 99.8%, of the fecal coliform counts by 99.2%, of the fecal streptococcus counts by 99.99%, of the 35°C standard plate counts by 96.5%, and of the 20°C standard plate counts by 93.7%. It must be emphasized however, that although these were significant reductions, there remained several thousand indicator organisms per 100 ml of effluent, and the likelihood that pathogenic bacteria, viruses, and parasites were also present, is high. As previously discussed, salmonella were isolated from

TABLE 15. DATA SUMMARY OF FINAL EFFLUENTS FROM PRIMARY TREATMENT FACILITIES OF RED MEAT PACKERS

	MF COUNT PER 100 ML				S.P.C. Per ML	
	Coliform	Fecal Coliform	Fecal Strep.	Ratio FC:FS	35°C	20°C
Median value	13,000,000	1,500,000	530,000	2.23	1,100,000	1,300,000
Geometric mean	14,000,000	1,700,000	640,000	2.41	1,000,000	1,000,000
Arithmetic mean	30,000,000	2,700,000	1,000,000	3.27	4,300,000	3,300,000
Minimum value	290,000	120,000	88,000	0.53	5,200	24,000
Maximum value	190,000,000	12,000,000	3,300,000	10.34	25,000,000	15,000,000

* values based on 17 sets of data.

TABLE 16. DATA SUMMARY OF FINAL EFFLUENTS FROM SECONDARY TREATMENT FACILITIES OF RED MEAT PACKERS

	MF COUNT PER 100 ML				
	Coliform	Fecal Coliform	Fecal Strep.	Ratio FC:FS	S.P.C. 35°C Per ML 20°C
Median value	47,000	11,000	4,000	2.04	97,000 100,000
Geometric mean	39,000	9,700	4,100	2.34	98,000 69,000
Arithmetic mean	120,000	28,000	23,000	2.94	310,000 120,000
Minimum value	3,200	700	200	0.75	17,000 8,400
Maximum value	770,000	180,000	180,000	6.52	1,300,000 310,000

* values based on 10 sets of data

the final effluents of this Calgary plant. Further study is needed to estimate the frequency and to quantitate the salmonella discharge. It would be of considerable interest and importance to do this because, for instance, Kenner et al. (1971) discovered, while investigating the efficiency of secondary treatment, that although fecal coliform numbers were reduced by 96%, *Pseudomonas aeruginosa* and *Salmonella* decreased by only 40% to 50% and 7% to 10% respectively.

5.2 Coliform and Fecal Coliform Identifications

A battery of biochemical tests were performed on a substantial number of coliforms and fecal coliforms isolated from the packing plant effluents. These were carried out in order to estimate the reliability of the MF procedures for the recovery of coliforms within the definition of a coliform as advocated by Bell and Vanderpost (1973), and to demonstrate the reliability of the coliform and fecal coliform tests for the isolation of these indicator organisms in meat packing plant wastes. Previously, MF techniques for the isolation of coliforms, using L.E.S. Engo Agar, have, in water samples from various sources, been shown to produce typical sheen colonies, which, when later phenotyped, were as much as 62% *Aeromonas* (Bell and Vanderpost, 1973). In addition, coliform and fecal coliform isolate studies from pulp and paper industries have demonstrated a high incidence of *Klebsiella* (Bordner et al., 1972). This has encouraged these industries to question the validity of the fecal coliform test as applied to pulp and paper mill wastes (Eickhoff, 1972).

For the present study, identification summaries for the coliform and fecal coliform isolates are shown in Tables 17 and 18. The MF procedures proved to be very reliable with 94.7% and 100% of the presumptive coliforms and fecal coliforms, respectively, fitting the typical biochemical identification schemes.

5.3 Salmonella Isolation Summary

Of 28 attempts (samples where no Moore swab placed, or Moore swab lost not included) to demonstrate the presence of *Salmonella* in both the plant treatment systems and the final effluents, there were 21 samples or 75% from which confirmed, positive isolations were made. The presence or absence

TABLE 17. IDENTIFICATIONS OF SELECTED COLIFORM ISOLATES FROM ALBERTA MEAT PACKING PLANT EFFLUENTS

Taxa	Number	Percentage
Escherichia coli	107	63.4
Citrobacter	9	5.3
Enterobacter	18	10.6
Klebsiella	26	15.4
Others	9	5.3
TOTAL	169	100.0

TABLE 18. IDENTIFICATIONS OF SELECTED FECAL COLIFORM ISOLATES FROM ALBERTA MEAT PACKING PLANT EFFLUENTS

Taxa	Number	Percentage
Escherichia coli	175	96.2
Enterobacter	4	2.2
Klebsiella	3	1.6
TOTAL	182	100.0

of salmonella in the waste treatment systems of each plant are summarized in Table 19. Of the 9 plants investigated (attempts where Moore swab lost not included), 7 or 78% produced final waste effluents which contained salmonella. This included the red meat packing plant in Calgary which has secondary treatment facilities. The salmonella serotypes isolated in the present study and the number of times each serotype was found are listed in Table 20. *Salmonella bredeney*, *S. kentucky*, *S. newington* and *S. senftenberg* were the most frequently isolated. Attempts to correlate the numbers of indicator bacteria with the presence or absence of salmonella in the samples failed, perhaps because there was not enough variation in the indicator organism counts. Such relationships have been demonstrated by Gallagher and Spino (1969), Geldreich (1970), Smith and Twedt (1971), Van Donsel and Geldreich (1971), and Smith *et al.* (1973). Geldreich (1970) suggested that although any projection of a qualitative recovery of salmonella, using fecal coliform counts as the indication, has limitations, 200 fecal coliforms/100 ml should be the limit for recreational waters.

In other investigations of Alberta meat packing plants, Dr. M. Finlayson, of the Public Health Laboratories of Alberta, has studied the occurrence of salmonella in poultry plants, beginning in 1971. She isolated 24 different serotypes over a four year period, and 11 of these were the same as ones isolated in the present study. These were *S. infantis*, *S. montevideo*, *S. tennessee*, *S. typhimurium*, *S. schwarzengrund*, *S. anatum*, *S. thompson*, *S. californica*, *S. organienburg*, *S. newington*, and *S. bredeney*.

Rhoda Laidley, of the National Enteric Reference Centre, Ottawa, has compiled a report (Appendix 1) containing a short history of the occurrences and known sources, ~~in~~ Canada, of the salmonella serotypes found in the present study. Also, this report presents a summary of the incidences of these serotypes, from both human and non-human sources, in Alberta and Canada for 1974. Of a total of 4,201 isolations in Canada, from all sources, of these serotypes, 544 or 13% were from Alberta, and of all salmonella serotype isolations in Canada in 1974, 10.3% were from Alberta.

5.4 Drug Resistance in Coliforms, Fecal Coliforms, and Salmonella from Alberta Meat Packing Plant Effluents

As discussed in the introduction, there is world wide concern over the increase in acquisition of drug resistance in bacteria, particularly in

TABLE 19. PRESENCE OF SALMONELLA IN ALBERTA MEAT PACKING
PLANT WASTE TREATMENT FACILITIES

		Within Treatment System	Final Effluent	Moore Swab Lost
<u>Red Meat Packers</u>				
Edmonton	1	+	+	
	2		+	
	3		+	
	4		+	
Red Deer	5		+	
	6		-	
	7			+
Calgary	8		-	
	9	+	+	
<u>Poultry Packers</u>				
Edmonton	1		+	
	2			+

TABLE 20. SALMONELLA SEROTYPES ISOLATED FROM WASTE TREATMENT FACILITIES OF ALBERTA MEAT PACKING PLANTS

SALMONELLA SEROTYPES	NUMBER OF SAMPLES SEROTYPE FOUND IN
S. alachua	4
S. anatum	2
S. bredeney	5
S. californica	4
S. derby	3
S. eimsbuettel	1
S. good	1
S. halmstad	2
S. infantis	2
S. java	1
S. kentucky	5
S. montevideo	4
S. newington	5
S. oranienburg	1
S. schwartzengrund	1
S. senftenberg	5
S. tennessee	1
S. thompson	2
S. typhimurium	1
S. typhimurium var copenhagen	3
Rough strains Sub genus I	2

pathogens such as *Pseudomonas*, *Salmonella*, and *Staphylococcus*, through conjugation with R factor - carrying organisms, for instance, *E. coli*. In the present study, there was concern that pathogens such as *Salmonella*, in meat packing plant effluents entering the municipal sewage lagoons of Edmonton, or the municipal treatment systems of Red Deer and Calgary, might come into contact with R factor - carrying *Pseudomonas*, *Klebsiella* and *E. coli* from human sewage. From this contact, a transfer of resistance would be possible. Furthermore, drug resistant enteric organisms in the packing plant effluents, originating from livestock given antiobiotic-treated feedstuffs, might transpose their resistance capacity to other organisms in the municipal facilities. Dr. M. Finlayson (personal communication) has stated that there exist resistant strains of *S. typhimurium* in Alberta which are involved in bovine infections. The result has been several cases of bovine illness and death in the past few years.

Since disinfection of treated sewage wastes is not policy in Alberta, there is a high probability of resistance transfer among bacteria in the sewage systems, before their subsequent release to the environment. Antibiotic resistances of the coliform, fecal coliform, and salmonella isolates tested in the present study are listed in Table 21.

5.4.1 Antibiotic Resistances of the Isolates. Three coliform isolates and no fecal coliforms or salmonella were resistant to Chloramphenicol. It is effective against both gram positive and gram negative bacteria and is preferentially used for the treatment of *Salmonella typhi* and other salmonella infections.

There were 81% coliforms, 90.9% fecal coliforms and 100% salmonella resistant to Erythromycin. This prevalence of resistance is not surprising since this antibiotic is similar to penicillin in range of antimicrobial activity, and is used mostly for gram positive infections.

High percentages of the coliforms (99%), fecal coliforms (95.5%) and salmonella (100%) were resistant to Novobiocin. This was to be expected since this drug is generally used to combat gram positive infections.

Similarly to Novobiocin, most coliform (99%), fecal coliform (95.5%)

TABLE 21. ANTIBIOTIC RESISTANCE OF 95 COLIFORM, 22 FECAL COLIFORM AND 20 SALMONELLA ISOLATES FROM ALBERTA MEAT PACKING PLANT EFFLUENTS

Antibiotic	Units	COLIFORMS		FECAL COLIFORMS		SALMONELLA	
		Number Resistant	% Resistant	Number Resistant	% Resistant	Number Resistant	% Resistant
Chloramphenicol	10 ug	3	3.2	0	0	0	0
Erythromycin	10 ug	77	81	20	90.9	20	100
Novobiocin	5 ug	94	99	21	95.5	20	100
Cloxacillin	5 ug	94	99	21	95.5	20	100
Penicillin G	1.5 units	95	100	22	100	20	100
Ampicillin	2 ug	53	56	17	77.3	0	0
Streptomycin	10 ug	6	6.3	1	4.5	0	0
Tetracycline	10 ug	24	25	3	13.6	0	0

and salmonella (100%) isolates were resistant to Cloxacillin. This semi synthetic penicillin is used principally against Gram positive bacteria.

All the coliforms, fecal coliforms, and salmonella tested were resistant to Penicillin G which is used almost exclusively against Gram positive bacteria.

Ampicillin, a semi synthetic penicillin, is used against Gram positive organisms such as *Staphylococcus*, *Pneumococcus* and Group A beta - hemolytic *Streptococcus*. However, it has been found to be effective against *Escherichia coli*, *Klebsiella*, *Shigella*, *Salmonella typhi* and other salmonella, *Proteus mirabilis*, and other susceptible Gram negatives. In view of this, it is of some interest to note that 56% of the coliform and 77.3% of the fecal coliform isolates were resistant to this antibiotic. There were no resistant salmonella.

Streptomycin is usually considered to have a similar range of antimicrobial activity to penicillin, and thus be most effective against Gram positives. In the present study, very small percentages of the coliforms and fecal coliforms (6.3% and 4.5% respectively), and none of the salmonella, were resistant to this drug.

Tetracycline is used against both Gram positive and Gram negative organisms. Although *E. coli*, *Aerobacter aerogenes*, *Shigella*, and *Klebsiella* are usually considered to be susceptible Gram negatives, in the present study, 25% of the coliforms and 13.6% of the fecal coliforms were resistant to Tetracycline. There were no resistant salmonella.

5.4.2 Summary for Antibiotic Resistance. There were several coliform and fecal coliform isolates which were resistant to those antibiotics generally considered to be effective against Gram negative bacteria, namely, Chloramphenicol, Tetracycline, and particularly Ampicillin (Table 21). Although none of the salmonella displayed resistance to these drugs, the inherent dangers of resistance transfer, if sensitive salmonella and resistant coliforms are permitted to coexist in non-disinfected waste, should be apparent. For example, Ampicillin and Chloramphenicol are the principal antibiotics used for the treatment of typhoid fever.

In Alberta (Dr. M. Finlayson, personal communication), biogroups of *S. typhimurium* from ducks and bovines have been found to be resistant to Streptomycin, Tetracycline, Ampicillin, and Sulpha Drugs.

5.5 Salmonella Survival in Water

The survival curves in Figure 4 demonstrate that *S. typhimurium*, in nutrient broth inoculated into 20 ℓ of water to give an initial concentration of 7.0×10^6 cells/ml, increased in numbers during the first 24 hour and then, very gradually, died off. After 12 days, there still remained 5.3×10^5 cells/ml. The revival of growth of the stationary phase culture upon initial entry into the 20 ℓ of water was probably due to the increase in oxygen availability, and possibly to a dilution of toxic substances produced during bacterial growth. It is conceivable that similar regrowth could occur for instance, when non-disinfected sewage treatment plant effluents are released to receiving water bodies.

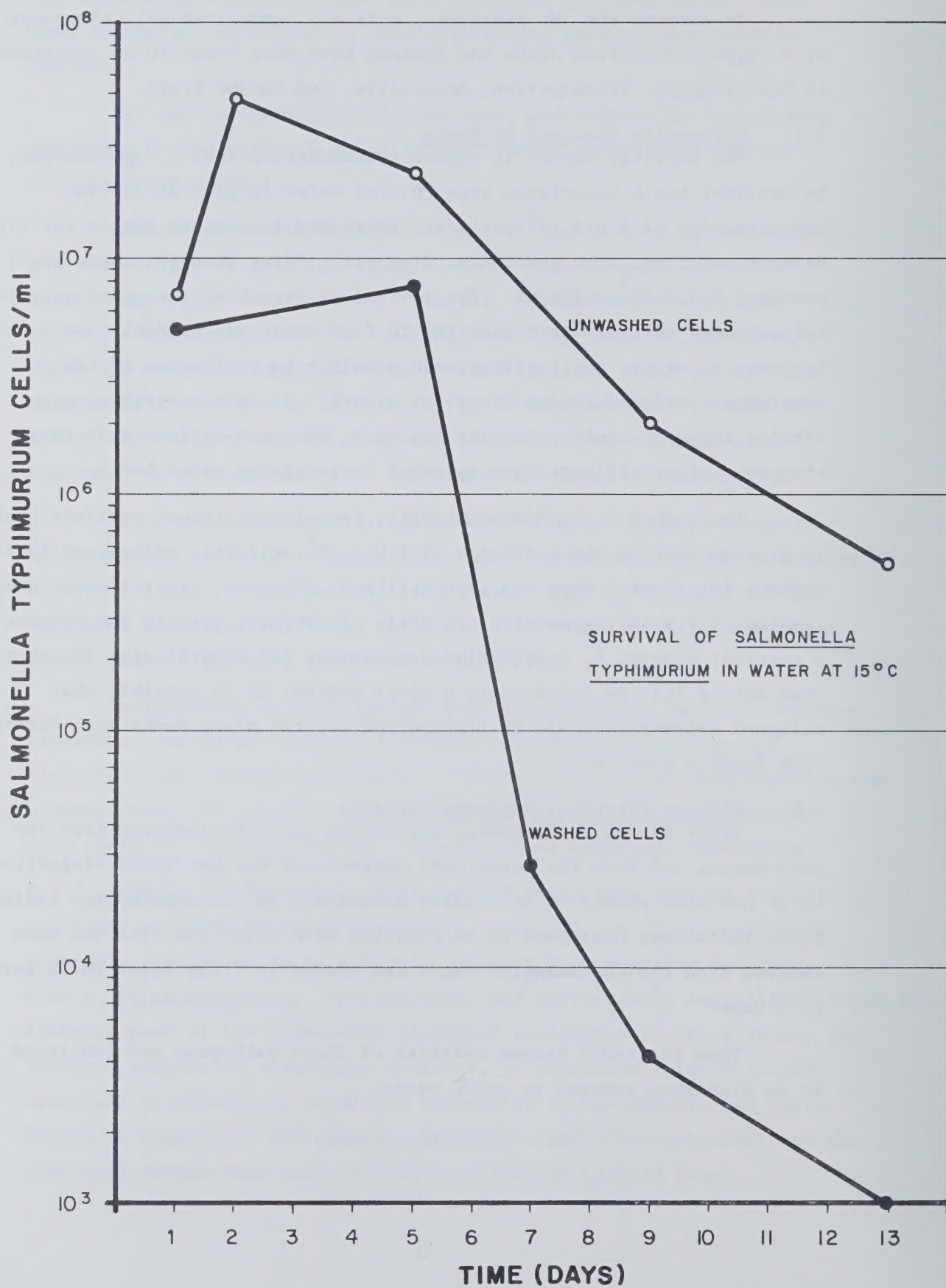
The washed *S. typhimurium* cells, inoculated without nutrient broth to give an initial concentration of 5.0×10^6 cells/ml, maintained their numbers for about 4 days and then declined. However, after 12 days there remained 8.7×10^2 salmonella cells/ml. Therefore, even in the absence of a nutrient supply, *S. typhimurium* can survive for several days in water. Considering this in relation to a river system, it is possible that released salmonella could be transported several miles downstream before they finally died out.

5.6 Fish as Potential Pathogen Carriers

After as few as 24 hours, salmonella could be isolated from the skin mucous and from the intestinal contents of Rainbow Trout fingerlings which had been placed in 15°C water containing 10^6 *S. typhimurium* cells/ml. These isolations continued to be possible even after the fish had been removed from the contaminated water and placed in fresh water for a period of 3 days.

Thus the trout became carriers of these pathogens and continued to be so even when removed to clean water.

FIGURE 4 SALMONELLA SURVIVAL IN WATER



REFERENCES

1. Ahuja, K.L., and Khera, X., "Isolation of Salmonella in Relation to Mortality and Carrier State in Pigs", Indian J. Exp. Biol., 10, 241 (1972).
2. Allen, N., and Pelczar, M.J., "Bacteriological Studies on the White Perch, *Roccus americanus*", Chesapeake Sci., 8, 135 (1967).
3. American Public Health Association, Standard Methods for the Examination of Water and Waste-Water, 13th Ed., Washington, D.C., (1971).
4. Anderson, E.S., "Transferable Drug Resistance", Sci. J., 4, 71 (1968).
5. Anderson, E.S., "The Ecology of Transferable Drug Resistance in the Enterobacteriaceae", Ann. Rev. Microbiol., 22, 131 (1968).
6. Anderson, E.S., Galbraith, N.W., and Taylor, C.E.D., "An Outbreak of Human Infection Due to *Salmonella typhimurium* Phage - Type 20a Associated with Infection in Calves", The Lancet, 1, 854 (1961).
7. Anderson, E.S., and Hobbs, B.C., "Studies on the Strain of *Salmonella typhi* Responsible for the Aberdeen Typhoid Outbreak", Israel J. Med. Sci., 9, 162 (1973).
8. Baldwin, R.A., "The Development of Transferable Drug Resistance in Salmonella and its Public Health Implications", J.A.V.M.A., 157, 1841 (1970).
9. Bell, J.B., and Vanderpost, J.M., "Comparison of Membrane Filtration Methods in the Isolation of Coliforms", Proc. 16th Conf. Great Lakes Res., Internat. Assoc. Great Lakes Res., 15 (1973).
10. Benarde, M.A., "Land Disposal and Sewage Effluent: Appraisal of Health Effects of Pathogenic Organisms", J.A.W.W.A., 65, 432 (1973).

REFERENCES

11. Berger, B.B., Jensen, E.C., Ludwig, H., Romer, H., Shapiro, M.A., Senn, C.L., "Coliform Standards for Recreation Waters", J.S.E.D., (A.S.C.E.) 89 (4), 57 (1963).
12. Bordner, R.H. et al., Proceedings of Seminar on the Significance of Fecal Coliforms in Industrial Wastes, N.T.I.S. PB-216-065, Denver, Colorado (1972).
13. Carpenter, J.A., Elliot, J.G. and Reynolds, A.E., "Isolation of Salmonellae from Pork Carcasses", Appl. Microbiol., 25, 731 (1973).
14. Clarenburg, A., Hemmes, G.D. and Wagenvoort, W., "On the Occurrence of *Salmonella bareilly* in Man and Animals and the Mode of Transmission on the Salmonellosis caused by this Species", Antonie van Leeuwenhoek, 18, 171 (1952).
15. Clark, G.M., Kaufmann, A.F., Gangarosa, E.J., and Thompson, M.A., "Epidemiology of an International Outbreak of *Salmonella agona*", The Lancet, II, 490 (1973).
16. Cowan, S.T., and Steel, K.S., Manual for the Identification of Medical Bacteria, Cambridge Univ. Press, London (1965).
17. Davidson, K.R., "*Salmonella dublin* Abortion in a New South Wales Dairy Herd", Australian Vet. J., 49, 174 (1968).
18. Durrani, S.M.A., "Animal wastes, Their Health Hazards and Treatment", Thai J. Agr. Sci., 4, 265 (1971).
19. Dutka, B.J., and Bell, J.B., "Isolation of Samonellae from Moderately Polluted Waters", J.W.P.C.F., 45, 316 (1973).
20. Edwards, P.R., "Salmonellosis: Observations on Incidence and Control", Annals N.Y. Acad. Sci., 70, 598 (1958).

21. Edwards, P.R., and Ewing, W.H., Identification of Enterobacteriaceae, 3rd Ed., Burgess, Minneapolis, Minnesota (1972).
22. Eickhoff, T.C., *Klebsiella pneumoniae* Infection: A Review with Reference to the Water-Borne Epidemiological Significance of *K. pneumoniae* Present in the Natural Environment, Stream Improvement Technical Bulletin No. 254. National Council of the Paper Industry for Air and Stream Improvement, New York, N.Y., (1972).
23. Feary, T.W., Sturtevant, A.B., and Lankford, J., "Antibiotic - Resistant Coliforms in Fresh and Salt Water", Arch. Environ. Health, 25, 215 (1972).
24. Finlayson, M., "Personal Communication", Alberta Public Health Laboratory, University of Alberta Campus, 114 St., Edmonton (1975).
25. Galbraith, N.S., Mawson, K.N., Maton, G.E., and Stone, D.M., "An Outbreak of Human Salmonellosis Due to *Salmonella saint-paul* Associated with Infection in Poultry", Public Health and Public Health Lab. Service, London, Monthly Bulletin, 21, 209, (1962).
26. Gallagher, T.P., and Spino, D.F., "The Significance of Numbers of Coliform Bacteria as an Indicator of Enteric Pathogens", Water Res., 2, 169 (1968).
27. Galton, M.M., MacKel, D.C., Lewis, A.L., Haire, W.C., and Hardy, A.V., "Salmonellosis in Poultry and Poultry Processing Plants in Florida", Am. J. Vet. Res., 16, 132 (1955).
28. Galton, M.M., Smith, W.V., McElrath, H.B., and Hardy, A.B., "Salmonella in Swine, Cattle and the Environment of Abattoirs", J. Infectious Diseases, 94, 236 (1954).
29. Gangarosa, E.J., Barker, W.H., Baine, W.B., Morris, G.R., and Rice, P.A., "Man v. Animal Feeds as the Source of Human Salmonellosis", Lancet, 1, 878 (1973).

30. Geldreich, E.E., "Applying Bacteriological Parameters to Recreational Water Quality", J.A.W.W.A., 62, 113 (1970).
31. Geldreich, E.E., "Water-Borne Pathogens", In Water Pollution Microbiology, Wiley and Sons, N.Y., Ed. Mitchel, R., p. 207 (1972).
32. George, J.T.A., Wallace, J.G., Morrison, H.R., and Harbourne, J.F., "Paratyphoid in Man and Cattle", Brit. Med. J., 3, 208 (1972).
33. Ghosh, A.C., "An Epidemiological Study of the Incidence of Salmonellas in Pigs", J. Hyg., Camb., 70, 151 (1972).
34. Guinee, P.A.M., "Bacterial Drug Resistance in Animals", Annals N.Y. Acad. Sci., 182, 40 (1971).
35. Habermalz, D., "A Report on Latent Salmonella Infection in German Cattle for Slaughter", Berl. Münch. Tierärztl. Wochenschr., 86, 206 (1973).
36. Haig, D.A., "Disease Hazard of Pathogenic Organisms in Farm Waste Material", J. Sci. Food Agric. 23, 795 (1972).
37. Hamm, D., "Characteristics of Effluents in Ten Southeastern Poultry Processing Plants", Poultry Science, 51, 825 (1972).
38. Hardy, A.V., and Galton, M.M., "Salmonellosis: The Role of Food Processing Plants in the Dissemination of *Salmonella*", Am. J. Tropical Med. Hyg., 4, 725 (1955).
39. Heard, T.W., Jennett, N.E., and Linton, A.H., "Changing Patterns of *Salmonella*" Excretion in Various Cattle Populations", Vet. Rec., 90, 359 (1972).
40. Hendricks, C.W., "Increased Recovery Rate of Salmonellae from Stream Bottom Sediments Versus Surface Waters", Appl. Microbiol., 21, 379 (1971).
41. Hobbs, B.C., "Microbiological Hazards of Meat Production", Food Manufacture, October, 29 (1974).

42. Hobbs, B.C., and Christian, J.H.B. (Eds.), "The Microbiological Safety of Food", Proc. Eighth Int. Symp. Food Microbiology, Adademic Press, London, New York (1973).
43. Huber, W.G., Korica, D., Neal, T.P., Schnurrenberger, P.R., and Martin, R.J., "Antibiotic Sensitivity Patterns and R Factors in Domestic and Wild Animals", Arch. Environ. Health, 22, 561 (1971).
44. Izzi, R., Rivellini, P., Guarino, C., and Urso, C., "Healthy Carriers of Salmonellae Among Animals Slaughtered in Naples", Igiene Moderna, 64, 125 (1971).
45. Jack, E.J., and Hepper, P.T., "An Outbreak of *Salmonella typhimurium* Infection in Cattle Associated with the Spreading of Slurry", Vet. Rec., 84, 196 (1969).
46. Jones, P.W., and Matthews, P.R.J., "Examination of Slurry from Cattle for Pathogenic Bacteria", J. Hyg. Camb., 74, 57 (1975).
47. Kampelmacher, E.H., and van Noorle Jansen, L.M., "Occurrence of *Salmonella* in Oxidation Ditches", J.W.P.C.F., 15, 348 (1973).
48. Kahle, H., and Gray, L., "Utilization and Disposal of Poultry By-Products and Wastes", Marketing Research Report No. 143, U.S. Dept. Agric., Washington, D.C. (1956).
49. Kenner, B.A., Dotson, G.K., and Smith, J.E., "Simultaneous Quantitation of *Salmonella* Species and *Pseudomonas aeruginosa*. I. Polluted Waters. II. Persistence of Pathogens in Sludge Treated Soils. III. Analysis of Wastes Treatment Sludges for *Salmonella* Species as a Surveillance Tool", Environ. Protection Agency Report No. PB-213 706, National Environmental Research Center, Cincinnati, Ohio (1971).

50. Kiser, J.S., Gale, G.O., and Kemp, G.A., "Antibiotics as Feedstuff Additives: The Risk-Benefit-Equation For Man", Critical Reviews in Toxicology (C.R.C.), 55 (1971).
51. Koditschek, L.K., and Guyre, P., "Resistance Transfer Fecal Coliforms Isolated from the Whippany River", Water Res., 8, 747 (1974).
52. Krishnaswami, S.K., "Health Aspects of Land Disposal Of Municipal Waste Water Effluents", Can J. Public Health, 62, 36 (1971).
53. Kucharski, B., "Bacteriological Examinations of Sewage of Representative Slaughterhouses in the Lublin District with a Special Consideration of Pathogenic Micro-organisms", Polski Archiwum Weterynaryjne, 13, 267 (1970).
54. Kumar, S., Saxena, S.P., and Gupta, B.K., "Carrier Rate of Salmonellas in Sheep and Goats and its Public Health Significance", J. Hyg., Camb., 71, 43 (1973).
55. Laidley, R., "Personal Communication", National Enteric Reference Centre, Department of National Health and Welfare, Ottawa (1975).
56. Lee, J.A., "Recent Trends in Human Salmonellosis in England and Wales: The Epidemiology of Prevalent Serotypes Other Than *Salmonella typhimurium*", J. Hyg., Camb., 72, 185 (1974).
57. Lee, H.A., Ghosh, A.C., Mann, P.G., and Tee, G.H., "Salmonellas on Pig Farms and in Abattoirs", J. Hyg. Camb., 70, 141 (1972).
58. Linton, A.H., Jennett, N.E., Heard, T.W., "Multiplication of *Salmonella* in Liquid Feed and Its Influence on the Duration of Excretion in Pigs", Res. Vet. Sci., 11, 452 (1970).
59. MacGregor, R.R., and Reinhart, J., "Person-to-Person Spread of Salmonella: A Problem in Hospitals?", Lancet ii, 1001 (1973).

60. Martin, ., "Occurrence and Survival of *Salmonella* in the Alimentary Tract of Some Freshwater Fishes", M. Sc. Thesis, Kansas State U., Manhattan, Kansas (1966).
61. Martineau, G., and Breton, J.P., "Typhoid Epidemic in the Village of Bouchette, Gatineau County, Quebec 1971", Epidemiological Bulletin, 16(4), 29 (1972).
62. McCaughey, W.J., McClelland, T.G., and Roddy, R.M., "Salmonella Isolations in Pigs", Vet Rec., 92, 191 (1973).
63. McDonagh, V.P., and Smith, H.G., "The Significance of the Abattoir in Salmonella Infection in Bradford", J. Hyg. Camb., 56, 271 (1958).
64. Miner, J.R., Fina, L.R., and Piatt, C., "*Salmonella infantis* in Cattle Feedlot Runoff", Appl. Microbiol., 15, 627 (1967).
65. Nemerow, N.L., "Poultry Processing Waste Treatment at Millsboro, Delaware", Proc. Ind. Waste Conf. Purdue Univ., 526 (1967).
66. Newell, K.W., McClarin, R., Murdock, C.R., MacDonald, W.N., and Hutchinson, H.L., "Salmonellosis in Northern Ireland with Special Reference to Pigs and Salmonella - Contaminated Pig Meal", J. Hyg., Camb., 57, 92 (1959).
67. Parsons, D., Brownlee, C., Wetter, D., Maurer, A., Haughton, E., Kornder, L., and Slezak, M., "Health Aspects of Sewage Effluent Irrigation", Pollution Control Branch, British Columbia Water Resources Service, Department of Lands, Forests, and Water Resources, Parliament Buildings, Victoria, B.C. April (1975).
68. Pfeiffer, K.R., "The Homestead Typhoid Outbreak", J.A.W.W.A., 5(65), 803 (1973).

69. Potter, L.F., and Baker, G.E., "The Role of Fish as Conveyors of Micro-Organisms in Aquatic Environments". Can J. Microbiol., 7, 595 (1961).
70. Prost, E., and Riemann, H., "Food - Borne Salmonellosis", Ann, Rev. Microbiol., 21, 405 (1967).
71. Quevedo, F., Dobosch, D., and Gonzalez, E., "Contamination of Horse Meat with Salmonellae: An Ecological Study. I. Working Horses", Gac, Vet., 35, 119 (1973).
72. Rowe, B., Giles, C., and Laing Brown, G., "Outbreak of Gastroenteritis Due to *Salmonella virchow* in a Maternity Hospital", Brit. Med. J., 3, 561 (1969).
73. Russell, R.G., "The Association of *Salmonella dublin* with Bovine Abortion in Victoria", Australian Vet. J., 49, 173 (1973).
74. Sethi, S.K., and Nath, M.L., "Prevalence of *Salmonella* species in Sheep and the Significance in Public Health", Indian J. Public Health, 12, 211 (1968).
75. Singh, S.P., Wesley, R.L., and Budd, E.A., "Characteristics of Poultry Processing Effluent", Poultry Science, 52, 1478 (1973).
76. Sinha, B.K., Roy, L.N. and Narayana, K.G., "Aerobic Microbes Isolated From Slaughtered Pigs and Their Public Health Importance", Indian J. Path. Bact., 157 (1970).
77. Skovgaard, N., and Nielsen, B.B., "Salmonellas in Pigs and Animal Feeding Stuffs in England Wales and in Denmark", J. Hyg., Camb., 70, 127 (1972).
78. Smith, R.J., and Twedt, R.M., "Natural Relationships of Indicator and Pathogenic Bacteria in Stream Water", J.W.P.C.F., 43, 2200 (1971).
79. Smith, R.J., Twedt, R.M., and Krusel Flanigan, L., "Relationships of Indicator and Pathogenic Bacteria in Stream Waters, J.W.P.C.F., 45, 1736 (1973).

80. Snieszko, S.F., "Remarks on Some Facets of Epizootiology of Bacterial Fish Diseases", Develop. in Ind. Microbiol., 5, 97 (1964).
81. Spino, D.F., "Elevated - Temperature Technique for the Isolation of Salmonellae from Streams", Appl. Microbiol., 14, 591 (1966).
82. Srivastava, S.K., Singh, V.B., and Singh, N.P., "Bacterial Flora of Poultry Environment", Indian J. Microbiol., 12, 7 (1972).
83. Sturtevant, A.B., and Feary, T.W., "Incidence of Infectious Drug Resistance Among Lactose - Fermenting Bacteria Isolated from Raw and Treated Sewage", Appl. Microbiol., 18, 918 (1969).
84. Sturtevant, A.B., Cassell, G.H., and Feary, T.W., "Incidence of Infectious Drug Resistance Among Fecal Coliforms Isolated from Raw Sewage", Appl. Microbiol., 21, 487 (1971).
85. Takacs, J., and Nagy, G., "Salmonella Occurrence in Products of Animal Origin in 1969-70", Magyar Általánosok Lapja, 28, 151 (1973).
86. Taylor, R.J., "A Further Assessment of the Potential Hazard for Calves Allowed to Graze Pasture Contaminated with *Salmonella dublin* in Slurry", Brit. Vet. J., 129, 354 (1973).
87. Toxopeus, R., Tennant, A.D., Bastien, J.A.P., Beauchamp, M., and Hayes, J.P., "Notes on Bacterial Pollution Indicators in a Liquid Manure System", Bacteriological Laboratories, Ottawa, Technology Development Branch, Water Pollution Control Directorate, E.P.S. February (1974).
88. Van Donsel, D.J., and Geldreich, E.E., "Relationships of Salmonellae to Fecal Coliforms in Bottom Sediments", Water Res., 5, 1079 (1971).

89. Watanabe, T., "I. Selected Methods of Genetic Study of Episome - Mediated Drug Resistance in Bacteria", Methods in Medical Research, 10, 202 (1964).
90. Watts, P.S., and Wall, M., "The 1951 *Salmonella typhimurium* Epidemic in South Australia", Australian Vet. J., 28, 165 (1952).
91. Weissman, M.A., and Carpenter, J.A., "Incidence of Salmonellae in Meat and Meat Products", Appl. Microbiol., 17, 899 (1967).
92. Williams, L.P., and Hobbs, B.C., "Enterobacteriaceae Infections", In Disease Transmitted from Animals to Man. 6th Ed., W.T. Hubert, W.F. McCulloch, and P.R. Schnurrenberger (Eds.), Charles C. Thomas, Springfield, Illinois (1974).
93. Williams, L.P., and Newell, K.W., "Salmonella Excretion in Joy-Riding Pigs", J. Public Health, 60, 926 (1970).
94. Williams Smith, H., "Incidence in River Water of *Escherichia coli* Containing R Factors", Nature, 228, 1286 (1970).
95. Woollen, A., "Salmonellosis is an Increasing World Problem", Food Manufacture, October, 3 (1974).

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Comparative Study of some serotypes of Salmonella isolates
from human and non-human sources in Alberta and Canada to
the same serotypes from plant effluents in Alberta.

by

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S. alachua (35:z4.z23:-) The first isolation of this serotype in Canada was from animal feed. In January 1969, this serotype was identified for the first time from humans in Alberta. Nine additional cases were reported from the same province in that year.

S. californica (4.5.12:m.t.-) During 1974, 12 of the 15 human cases and 24 of the 25 non-human isolations in Canada were from Alberta (Table 1).

S. eimsbuettel (6.7.14:d:l.w) The first report of this serotype in Canada was from a human in 1964. Coconut has been recognized as one source for this serotype and it is now found in a wide variety of animal feeds (Table 2).

S. good (21:f,,g:e,n.x) The first identification of this serotype in Canada was from a margay cat and since that time has been reported only rarely. In the plant effluent survey this serotype was isolated from a horse meat plant effluent in 1975. S. good is a common serotype in South American countries e.g. Argentina and Peru.

S. halmstad (3,15:g,s,t:-) This serotype has been identified mainly from animal feeds in Canada.

S. infantis (6,7:r:l.5) In Canada, over the past two years, it ranks among the top five most common serotypes from both human and non-human sources. Poultry and poultry environment are the predominant non-human sources, followed by animal feeds (Table 2).

S. montevideo (6.7:g.m.s.:-) This serotype appears among the top ten most common serotypes in Canada from human and non-human sources over the past two years. Poultry, poultry environment and animal feeds are the most common sources of non-human isolations (Table 2).

S. newington (3.15:e,h:1.6) S. oranienburg (6.7:m.t:-) and S. schwarzengrund (1,4.12,27:d:1.7) These serotypes are not among the top five serotypes in Canada and only appear among the top ten under special circumstances (outbreak, or large specific type of survey). Poultry, poultry environment and animal feeds are the more common sources (Table 2).

S. tennessee (6,7:z29:-) This serotype is often isolated from animal feeds (Table 2).

S. senftenberg (1.3.19:g,s.t:-) This serotype has been more frequently reported over the past year from both human and non-human sources. Animal feeds are a common source of isolation of this salmonella and over the past year several isolations were from a survey of gelatin and its environment (Table 2).

S. thompson (6,7:k-1.5) S. thompson is a common serotype in Canada and ranks among the top five serotypes isolated from both human cases and non-human sources. Poultry and poultry environment are predominant sources of isolation of this serotype (Table 2).

S. typhimurium (1,4,5,12:i:1.2) The most common serotype, from both cases and non-human sources, in Canada is S. typhimurium. Among non-human sources this serotype is common among farm live stock especially cattle but is frequently isolated from poultry and poultry environment, horses, cats, dogs, pigs and sheep. S. typhimurium is isolated from a wide variety of birds, both tame and wild. It seems to be the main serotype isolated from the wild animal population.

S. OR:r-1.5 These cultures were subgenus I salmonella of which the O antigens were not identifiable due to roughness. The most likely serotype to which they might belong is S. infantis. A number of such strains were isolated from chicken litter (Table 2).

S. OR:g,s,t:- These cultures are subgenus I salmonella strains of which the O antigens are not identifiable due to roughness, but the most likely serotype would be S. senftenberg. Such strains were isolated from plant effluents early in 1975.

TABLE 1. SALMONELLA SEROTYPES ISOLATED FROM PACKING PLANT EFFLUENT SAMPLES, EPS, NORTHWEST REGION, BY INCIDENCE IN HUMAN AND NON-HUMAN SOURCES, ALBERTA AND CANADA, 1974

SEROTYPE	ALBERTA		CANADA	
	Human	Non-Human	Human	Non-Human
S. alachua		10	1	10
S. anatum	3	1	36	25
S. bredeney	1	2	17	14
S. californica	12	24	15	25
S. derby	5	4	78	14
S. eimsbuettel		3	3	25
S. good				1
S. halmstad				
S. infantis	15	34	302	117
S. java	8		85	28
S. kentucky	1	25	2	28
S. montevideo	8	20	327	83
S. newington	2	15	5	32
S. oranienburg	4	19	61	33
S. schwarzengrund			14	38
S. senftenberg	1	3	26	62
S. tennessee	1		11	17
S. thompson	23	24	264	87
S. typhimurium	190	85	1,643	624
S. virchow			6	23
S.OR:r-1.5		1		17
S.OR:g,s,t-				
Sub-total	274	270	2,898	1,303
All serotypes	443	340	5,308	2,303

TABLE 2(a). SALMONELLA SEROTYPES ISOLATED FROM PACKING PLANT EFFLUENT SAMPLES, EPS, NORTHWEST REGION; NON-HUMAN SOURCES IN ALBERTA AND CANADA, 1974

Serotype	Source	ALBERTA		CANADA	
		by Source	Total	by Source	Total
<u>S. alachua</u>	crackings	1		1	
	plant - pre-catch	1		1	
	manure				
	" -pre-catch fat	3	10	3	10
	" -pond inlet	4		4	
	treated effluent	1		1	
<u>S. anatum</u>	chicken			1	
	turkey			4	
	hawk	1		1	
	turtle and envir.			1	
	cod - smoked black		1	2	25
	salmon - smoked			4	
	scrap gelatin			8	
	rendered product			1	
	beach samples			2	
	river water			1	
<u>S. bredeney</u>	chicken			1	
	turkey			1	
	imp. frog legs			1	
	imp. snails		2	1	
	meat meal			5	14
	poultry truck litter			2	
	porcine			1	
	treated effluent	1		1	
	river water	1		1	
<u>S. californica</u>	chicken carcass			1	
	poultry plant - chill tank	1		1	
	- evisc. tank	1		1	
	poultry envir.	1		1	
	" dust	1		1	
	" litter	2	24	2	25
	" nest	2		2	
	" truck litter			3	
	river water	5		5	
	lagoon	3		3	
	cracklings	2		2	
	meat meal	3		3	

TABLE 2(b). SALMONELLA SEROTYPES ISOLATED FROM PACKING PLANT EFFLUENT SAMPLES, EPS, NORTHWEST REGION; NON-HUMAN SOURCES IN ALBERTA AND CANADA, 1974

Serotype	Source	ALBERTA		CANADA	
		by Source	Total	by Source	Total
<u>S. derby</u>	chicken			1	
	pressed pork			1	
	porcine			1	
	gelatin plant - celite bag			1	
	dog	1		1	
	dog meal	1	4	1	14
	meat meal			1	
	hog mix. suppl.			1	
	poultry food			4	
	meat plant envir.	2		2	
<u>S. cimsbuettel</u>	feather meal			4	
	fish meal			16	
	rendered product		3	1	25
	river water	1		1	
	lagoon	2		2	
	not stated			1	
<u>S. good</u>	river water			1	1
<u>S. halmstad</u>			0		0
<u>S. infantis</u>	avian			3	
	chicken	1		32	
	chicken meat, deboned			1	
	poultry			5	
	poultry meat			4	
	turkey	1		1	
	egg - processed			1	
	egg white, past.			1	
	liver - pork			1	
	minced beef			1	
	ground beef and soya-burger			3	
	hamburger			2	
	sandwiches			2	
	food - not spec.			1	
	poultry - water			4	
	" - chill tank	3		3	
	" - evisc. tank	4		4	
	" - feather tank	2		2	

TABLE 2(c). SALMONELLA SEROTYPES ISOLATED FROM PACKING PLANT EFFLUENT SAMPLES, EPS, NORTHWEST REGION; NON-HUMAN SOURCES IN ALBERTA AND CANADA, 1974

Serotype	Source	ALBERTA		CANADA	
		by Source	Total	by Source	Total
<u>S. infantis</u> (cont'd)	poultry - floor drain	7		7	
	" - feather drain	5		5	
	" - nest				
	" - barn dust	1		1	
	" - litter			10	
	meat meal	1		4	
	meat and bone meal			2	
	calf ration			1	
	poultry feed			1	
	steer feed			1	
	milk replacer			1	
	fish meal			5	
	river water	5		5	
	treated effluent	1		1	
	sewage	1	34	1	117
	broiler manure			1	
	dog			1	
	rabbit			1	
	not stated	1		2	
<u>S. java</u>	turtle and turtle envir.			25	
	turkey litter		0	1	28
	imp. frog legs			2	
<u>S. kentucky</u>	meat scraps	1		1	
	meat meal	6		7	
	imp. meat meal	1	25	1	28
	hog suppl.			1	
	meat plant - fat tank	8		8	
	treated effluent	9		9	
<u>S. montevideo</u>	purification plant			1	
	chicken			3	
	eggs (whole and powdered)			26	
	chicken salad			2	
	food - n/s			1	
	skim milk powder			1	
	poultry - chill tank	2		2	
	- water			2	
	- feather drain	1		1	
	- floor drain	3		3	

TABLE 2(d). SALMONELLA SEROTYPES ISOLATED FROM PACKING PLANT EFFLUENT SAMPLES, EPS, NORTHWEST REGION; NON-HUMAN SOURCES IN ALBERTA AND CANADA, 1974

Serotype	Source	ALBERTA	CANADA	Total
		by Source	by Source	
<u>S. montevideo</u> (cont'd)	poultry - evisc. tank	1	1	
	- giblets		2	
	- feathers		1	
	- nesting		6	
	- litter		1	
	- feed		6	
	blood meal	1	5	
	fish meal		2	
	meat meal		1	
	meat and bone meal	2	2	
	meat plant - fat tank	3	3	
	" " - treated effluent	3	3	
	cats and kittens	4	4	
	turtle and envir.		3	83
	not stated		2	
<u>S. newington</u>	chicken	1	1	
	poultry		1	
	hatchery fluff	1	1	
	poultry plant - evisc. tank	1	1	
	- floor drain	1	1	
	plant - envir.	2	2	
	- water		2	
	- floor		1	
	- ceiling		1	
	meat plant - envir.	2	2	32
	- fat tank	1	1	
	treated effluent	3	3	
	fish meal		9	
	" " reprocess.		1	
	" " cold dryer		1	
	meat meal	1	1	
	meat and bone meal	1	1	
	scrap meal		1	
	sow supplement	1	1	

TABLE 2(e). SALMONELLA SEROTYPES ISOLATED FROM PACKING PLANT EFFLUENT SAMPLES, EPS, NORTHWEST REGION, NON-HUMAN SOURCES IN ALBERTA AND CANADA, 1974

Serotype	Source	ALBERTA		CANADA	
		by Source	Total	by Source	Total
<u>S. oranienburg</u>	bovine	1		1	
	turtle and envir.			11	
	poultry plant - chiller	2		2	
	- floor drain	1		1	
	- feather drain	2		2	
	- evisc. tank	1		1	
	hatchery fluff	1		1	
	poultry litter	1	19	1	33
	fish meal			1	
	meat meal	4		4	
	imp. meat meal	1		1	
	meat and bone meal			1	
	cracklings			1	
	poultry protein suppl.	1		1	
	porcine feed	1		1	
	tankage	2		2	
	treated effluent	1		1	
<u>S. schwarzengrund</u>	chicken			5	
	poultry litter			19	
	meat and bone meal			1	
	gelatin plant - powder			6	38
	- cleaner			4	
	- scrapings			2	
<u>S. senftenberg</u>	not stated			1	
	eggs			1	
	chocolate balls			1	
	gelatin plant - powder			11	
	- scrap			14	
	- dryers			12	
	poultry - plant water			1	
	- feathers			2	
	- envir.			2	
	water		3	5	62
	blood meal			1	
	fish meal			2	
	meat meal			5	
	meat and bone meal			1	
	hog suppl.			1	
	lagoon	3		3	

TABLE 2(f). SALMONELLA SEROTYPES ISOLATED FROM PACKING PLANT EFFLUENT SAMPLES, EPS, NORTHWEST REGION; NON-HUMAN SOURCES IN ALBERTA AND CANADA, 1974

Serotype	Source	ALBERTA		CANADA	
		by Source	Total	by Source	Total
<u>S. tennessee</u>	egg powder			1	
	plant envir.			6	17
	blood meal			7	
	feather meal			2	
	not stated			1	
<u>S. thompson</u>	chicken			27	
	turkey			1	
	poultry giblets			14	
	" envir.	5		5	
	" nest	1		1	
	" litter	4		4	
	" clean litter	1		1	
	" crates			1	
	poultry plant - chill tank	8		8	
	- floor drain	1		1	
	- evisc. tank	2		2	
	eggs			2	
	canine			1	
	chinchilla			1	
	equine			2	
	elk		24	1	
	monkey			2	
	hospital floor	2		2	87
	not stated			3	
	imp. frog legs			2	
	turtle and envir.			6	
<u>S. typhimurium</u>	avian			4	
	bovine	2		258	
	chicken	10		39	
	duck			4	
	goose	1		5	
	turkey			15	
	poultry			20	
	chicken-necks			1	
	" -retail			4	
	poultry - carcass			38	
	" - giblets			8	
	" - water			1	
	" - envir.	4		4	
	" - litter	1		17	
	" - nesting			1	

TABLE 2(g). SALMONELLA SEROTYPES ISOLATED FROM PACKING PLANT EFFLUENT SAMPLES, EPS, NORTHWEST REGION; NON-HUMAN SOURCES IN ALBERTA AND CANADA, 1974

Serotype	Source	ALBERTA		CANADA	
		by Source	Total	by Source	Total
<u>S. typhimurium</u> (cont'd)	turkey litter			1	
	feather sample			6	
	hatchery	1		1	
	egg shell	1		1	
	poultry plant - water			1	
	- chill tank	19		19	
	- evisc. tank	2		2	
	- feather tank	1		1	
	- feather drain	8		8	
	- floor drain	11		11	
	chicken meat, processed			2	
	processed meat			3	
	minced beef			1	
	hamburg			2	
	raw sausage - beef and pork			1	
	meat block			1	
	ice machine			1	
	ice cream			1	
	fish cakes			1	
	imp. frog legs			3	
	thyme, imp.			1	
	food sample			1	
	horse meat			2	
	equine			33	
	canine			7	
	feline			7	
	lapine			3	
	ovine			3	
	porcine			6	
	canaries - imp.	1		3	
	Bohemian waxwings			1	
	finches - imp.			1	
	parrot - imp.			2	
	parakeet - imp.	1		2	
	pigeon	4		10	
	gull			5	
	imp. tree duck			1	
	imp. black bunting			1	
	sparrow			7	
	boa constrictor	1		1	

TABLE 2(h). SALMONELLA SEROTYPES ISOLATED FROM PACKING PLANT EFFLUENT SAMPLES, EPS, NORTHWEST REGION; NON-HUMAN SOURCES IN ALBERTA AND CANADA, 1974

Serotype	Source	ALBERTA		CANADA	
		by Source	Total	by Source	Total
<u>S. typhimurium</u> (cont'd)	mink - int.			1	
	moose	2		2	
	mouse - wild			1	
	orang (zoo)	2		2	
	ox	5		5	
	tiger		85	1	624
	meat plant - envir.	6		6	
	- fat tank	1		1	
	treated effluent	1		1	
	landfill leachate			1	
	cat and dog food			1	
	water			7	
	not stated			7	
<u>S. virchow</u>	imp. frog legs			22	
	not stated			1	23
<u>S. OR:r-1.5</u>	chicken litter	1	1	17	17

SALMONELLA IN POULTRY
PACKING PLANT EFFLUENTS

by

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ABSTRACT

Concern that salmonellae associated with poultry production in Ontario may be recycled through the environment by the discharge of inadequately-treated waste effluents led to the conduct of three case studies of poultry processing plants in 1975. Salmonellae of various serotypes were shown to be present in raw and treated wastes at three large, well-operated plants. Data presented support the need for effective treatment and disinfection of such wastes if the problem of poultry-associated salmonellosis is to be contained. The incidence of antibiotic resistance among fecal coliforms and salmonellae isolated from the waste streams was evaluated. Methods for the isolation of salmonellae, as selected on the basis of comparative data obtained, are proposed for use in waste treatment and effluent quality case studies of poultry plant operations.

RESUME

Une sérieuse inquiétude née du fait que les salmonellae associées à la production de la volaille en Ontario puissent être recyclées dans l'environnement par la décharge d'effluents inadéquatement traités, a conduit à l'étude de trois plants de transformation dans l'industrie de la volaille en 1975. Les effluents bruts et traités de trois plants importants et bien organisés ont démontré la présence de salmonellae de divers sérotypes. Les résultats présentés confirment la nécessité d'un traitement efficace avec désinfection de tels déchets pour assurer le contrôle de la salmonellose associée à la production de la volaille. L'incidence de la résistance aux antibiotiques de certains coliformes fécaux et salmonellae isolés des cours d'eau receveurs a été évaluée. Des méthodes pour l'isolation des salmonellae, choisies selon une étude comparative des résultats obtenus, sont proposées pour utilisation dans les études de contrôle de la qualité du traitement des déchets et des effluents des plants de transformation de la volaille.

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CONCLUSIONS

On the basis of data presented and discussed, it may be concluded that:

1. A world-wide association of salmonellae and poultry production has been clearly demonstrated during recent years; the problem is briefly reviewed and discussed in this Report;
2. Salmonellae were present in raw and treated liquid wastes from three large, well-operated Ontario poultry packing plants. The presence of these pathogenic bacteria in effluents discharged to the environment should not be permitted;
3. Effective chlorination of treated effluent before discharge, as observed at one processing plant, appeared to eliminate salmonellae from the effluent. Further study of chlorination efficiency, and of alternative methods of disinfection, including ozonation, is required.
4. Routine surveillance studies of poultry processing plant waste treatment and effluent quality should include tests for the presence of salmonellae, as well as for Pseudomonas aeruginosa densities and for the standard pollution index bacterial parameters; proposals are made for Salmonella isolation and identification methods for use in such routine studies;
5. Presumptive evidence is presented to show that the presence of R^+ fecal coliforms, multiply-resistant to three or more antibiotic drugs, could theoretically induce R factor transfer to salmonellae and other enterobacteriaceae in poultry processing wastes.

RECOMMENDATIONS

It is recommended that:

1. Disinfection of poultry processing plant wastes be required before discharge of the treated wastes to receiving streams or to land;

2. The Environmental Protection Service support studies designed to assess, and establish effective procedures for, the treatment and disinfection of poultry processing plant wastes which will eliminate the discharge of effluents containing salmonellae to the environment.

1 INTRODUCTION

The current status of salmonellae as a major cause of human illness, of domestic poultry as an important reservoir of salmonellae, and of poultry and poultry products as a vehicle for transmission of Salmonella to humans, is readily apparent from the scientific literature, which will be briefly reviewed in this Report. Surveys by the Department of National Health and Welfare since 1970 have shown a steady increase in the number of Salmonella-positive poultry reaching the consumer market. The Interdepartmental Salmonella Committee, with representatives from Health and Welfare Canada, Agriculture Canada, and Environment Canada, was reactivated in 1974 to resume the attack on salmonellosis in Canada.

A prime function of the ISC is to formulate methods and regulations that would ensure a reduction of salmonellosis in human populations and food chains. Emphasis has been given to the necessity of controlling the presence and propagation of salmonellae in the poultry industry. The Committee has identified animal feeds and breeder flocks as primary focal points of Salmonella transmission; it has also recognized that the processing of contaminated birds, and poor sanitation practices in processing plants, can disseminate these pathogens throughout market poultry products, and can be a major factor in recycling salmonellae through the external environment via untreated or inadequately treated waste discharges. Efficient control of salmonellae in poultry can only result from coordinated action by all control agencies, which would provide remedial action to all links in the infection chain.

More than 1,400 Salmonella serotypes have been identified; all, in general, are equally pathogenic to man and most domestic and wild animals and birds. Notable exceptions are S. typhi which causes typhoid fever in man, and S. pullorum and S. gallinarum which are responsible for extensive morbidity and mortality in flocks of domestic poultry. The degree of control applied to salmonellae depends on the seriousness of the disease produced. S. typhi is rigorously controlled because it may cause a substantial loss of human life, while S. pullorum and S. gallinarum are controlled because they kill poultry, and the costs to the industry would be excessive if they were not controlled. The incidence of S. infantis as an avian pathogen has increased significantly in recent years; the situation may warrant particular attention if the current trend continues. With the exception of these few serotypes, there has been little interest in the control of salmonellae except among those who are concerned with the safety of food and water (31).

In view of the world-wide association of salmonellae with the poultry industry, it is surprising that there has been a lack of interest in, and concern about, the discharge of these pathogens in processing wastes. Only two major effluent-evaluation studies have come to our attention. Hoadley et al. (20) conducted an excellent two-year study of waste streams at a chicken processing plant in Georgia, and Vanderpost and Bell (43) of the Environmental Protection Service, Northwest Region, recently reported data from a major study of red meat and poultry processing plant wastes in Alberta. Both studies identified a serious potential problem of environmental contamination and public health hazard associated with Salmonella-positive plant wastes, and recommended more-complete treatment, with disinfection of final effluents. Vanderpost and Bell also recommended that

further assessments be undertaken in other Regions to establish the extent of the problem in Canada.

The present study was undertaken with a two-fold purpose:

1. To determine the extent to which large, well-operated government-inspected poultry processing plants serve as reservoirs of salmonellae which could reach the external environment with inadequately disinfected effluents; and

2. To evaluate previously selected methods for the isolation of salmonellae, and to assess the possible applicability of the various methodological options to routine monitor studies of poultry plant effluent discharges.

Salmonellae isolated from treated and untreated wastewaters at three Ontario processing plants were identified to serotype through the application of biochemical and serological tests, and were subjected to antibiotic resistance screening. Other pollution-index bacteriological and chemical tests were applied to the same samples. For evaluation purposes, the battery of tests for the isolation of salmonellae included comparisons of: a modified Moore Swab technique, and the filtration of one-liter grab samples; three enrichment broths; incubation of enrichment cultures at two temperatures (37° and 41.5°C) for 24 and 48 hours; and three plating agars. This test battery is too time and labour intensive for routine application. The intent was to provide data which would permit the selection of one or two isolation pathways which would reliably demonstrate the presence of salmonellae in contaminated effluents, and which could be used routinely in future case studies.

LITERATURE REVIEW

Woollen (47) stated in 1974 that, "salmonellosis is an increasing world problem". In England and Wales, the number of cases of human salmonellosis doubled between 1966 and 1971 (23). In the U.S.A., about two million human salmonella infections are reported each year (32); related losses to the American economy are estimated at about 100 million dollars annually.

In Canada, it is estimated that about five thousand diagnosed cases of human salmonellosis occur annually. It is known, however, that salmonella infections are grossly unreported, and that diagnosed cases may represent only about one per cent of the true total; there may therefore be close to five hundred thousand salmonella infections among the population.

A general increase in the number of reported salmonellosis outbreaks has resulted in intensified efforts to identify and control environmental sources of salmonellae. Poultry and poultry products have been identified as a prime source of Salmonella food poisoning in man.

Salmonellae were responsible for more than half of the reported cases of food-borne illnesses in the U.S. in 1969 (7). It was estimated (5) that poultry and poultry products accounted for 50.1 per cent of all non-human isolates of salmonellae in the U.S. in 1972. In Canada, the statistics on reported Salmonella infections and outbreaks are somewhat limited, but a conservative estimate would be more than 200,000 cases per year (4). Poultry and poultry products are responsible for nearly half of the common-vehicle epidemics reported.

A study of Surkiewicz et al. (37) of nine federally-

inspected U.S. chicken evisceration plants revealed that more than 20 per cent of eviscerated chicken samples were positive for salmonellae. Sadler et al. (33) reported very similar findings in a study of eight processing plants. Wilder and MacCready (45) investigated Salmonella contamination at the processing plant and retail level in Massachusetts; 50.2 per cent of 237 retail samples of dressed poultry were positive for Salmonella. Half of these were from government-inspected plants. They concluded that salmonellae do not live and multiply in the processing plant environment but are constantly reintroduced by contaminated birds entering the plant. When sanitation and processing procedures are poor and inadequate, cross-contamination occurs. Similar data were reported by other investigators (10,25,46).

Studies by a number of investigators, including Morris et al. (28) have identified three key stages in poultry production and processing to which control of Salmonella infection should be applied:

1. Animal by-products in feeds. Animal feeds are frequently contaminated with salmonellae before, during or after processing. The importance of feeds as a major source of infection of meat products has been stressed in the literature (19,32,34). There is considerable evidence to show that, under certain conditions, self-sustaining cycles of infection independent of the original source, can become established (21).

2. Salmonella-infected breeder flocks can be an important source of salmonellae in poultry.

3. Poor sanitation and unsatisfactory techniques in processing plants frequently lead to the spread of salmonellae throughout the plant and the product.

2.1 Salmonellae in Poultry Plants

Morris and Wells (29), in a two-year study of a poultry processing plant, showed that salmonellae were spread from flock to flock during the serial processing of birds from different sources; contamination was reduced by in-plant washing procedures, but recontamination of the carcasses occurred particularly during the evisceration and chilling processes.

Nivas et al. (30) studied three turkey processing plants and showed that two were salmonellae-free before the day's operations began. Serotypes spread through the plants were the ones introduced by the poultry flocks processed. Salmonella contamination tended to be heaviest on defeathering machines. They concluded that the incidence of salmonellae could be lowered by using steam instead of water for scalding birds, and by in-plant chlorination of water in the final washer at 20 mg/l before the carcasses entered the chiller tank, or chlorination at 200 mg/l in the chiller tank.

Although chlorination of chill tanks appears to be a promising Salmonella control measure, its use cannot be entertained in Canada until adequate data are collected to prove its efficiency, and until the absence of a chlorine-mediated production of carcinogens is proven. In 1975, the U.S. poultry industry petitioned the Food and Drug Administration to permit poultry products to be exposed to water containing not more than 50 mg/l free available chlorine upon entering the system (6). Chlorination approval is considered important because the use of chlorine plays a key role in the industry's program to combat Salmonella infection. The petition gained the support of the U.S. Department of Agriculture, which recommended that chlorine be used

in all processing plants. The FDA recognizes that the problem is more a legal one than a safety one; toxicologists will study all available data to determine if a potential health problem is associated with in-plant chlorination.

2.2 Salmonellae in Plant Effluents

Hoadley et al. (20) conducted a two-year study of a single poultry processing plant handling about 80,000 chickens per day. Populations of salmonellae and indicator bacteria were estimated in the raw wastes, through waste treatment, and in the waters of the receiving stream. They demonstrated that salmonellae were present in poultry processing wastes in a surprisingly constant ratio to fecal coliforms (in excess of one Salmonella per 500 fecal coliforms), but that serotypes in the plant environment are constantly changing. Hoadley et al. concluded that poultry processing wastes are a possible source of infection among human and animal residents of the environment, and recommended disinfection of poultry processing wastes.

Vanderpost and Bell (43), in a report of a major study of effluents from Alberta red meat and poultry processing plants, showed that such effluents are important sources of coliforms, fecal coliforms, fecal streptococci, and salmonellae. Seven of the nine plants studied were shown to discharge Salmonella-contaminated effluents; salmonellae were frequently recovered from very small (10.0 and 1.0 ml) samples of effluent. Twenty-one different Salmonella serotypes were identified during the study. Vanderpost and Bell recommended further treatment of packing plant wastes, including disinfection of final effluents. Where possible, the dissemination of salmonellae throughout the environment should be stopped; regulations prohibiting the discharge of these organisms in packing plant effluents are required.

2.3 Environmental Reservoirs of Salmonellae

Contributions of the environment (rodents, wild birds, insects and water) to the bacterial contamination of poultry flocks and processing plants should not be overlooked or minimized when control measures are under consideration. For example, Anderson (3) described a study of a fish meal plant in which S. newington was isolated from rat feces, flies, and one of eleven seagulls, as well as from swabs from roof beams and various other surfaces throughout the plant and from the fish meal produced. No other Salmonella serotype was found.

There is no question that domestic and wild animal and bird populations constitute important reservoirs of enteric disease; the presence of enteric pathogens in animal and bird feces is well documented (26,27,36,38). The degree of public health hazard associated with the entry of potentially-pathogenic fecal material from these sources into surface waters is difficult to evaluate, but inevitably there will be some recycling of these bacteria through the environment. Cherry et al. (9) isolated salmonellae from a variety of Georgia streams considered as minimally polluted or free from domestic animal and human contamination. Similarly, Fair and Morrison (14) showed that Salmonella and Arizona serotypes, presumably derived from the feces of wild animals, were present in supposedly high-quality mountain stream water. The public health significance of warm-blooded animal pollution, and the transport of microorganisms to streams, has recently been reviewed by Geldreich (16). In a previous study by this Laboratory (39), evidence was presented to show that the wild animal and bird populations of a suburban watershed probably were the source of salmonellae isolated from drainage streams.

2.4 Drug Resistance Transfer

Resistance to chemotherapeutic agents can be transferred, from one bacterium to another, by R factors. R factors are extra chromosomal elements of nucleic acid (episomes) which can be transferred, either in vivo or in vitro, between Gram-negative bacteria by means of conjugation and transduction. Details of specific mechanisms involved in the transfer process have been cited and discussed in the literature (2, 8,44); the problems presented by resistance transfer, and the public health implications of this phenomenon in sewage polluted water have been thoroughly reviewed by Grabow et al. (17), and will be only briefly outlined here.

Two factors appear to be involved in the spread of R^+ bacteria. Drug therapy in humans and animals, and drug utilization in animal husbandry, selects for, but does not create, resistance plasmids. Secondly, R^+ bacteria are transferred from excretors to the population at large; sewage may be of prime importance in this regard. There may be a resulting environmental increase in the incidence of bacteria which show high level resistance to many drugs, and which may also be resistant to other antibacterial agents such as ultra violet light, heavy metals and bacteriophages; the virulence and infectivity of pathogens may also be enhanced. Intestinal Gram-negative bacteria like coliforms may act as reservoirs of R factors and transfer them to pathogens. Grabow et al. presented evidence that sewage polluted water may play an important role in the spread of coliform and other bacteria carrying R factors, and called for a re-evaluation of water quality standards and for more advanced purification of sewage prior to discharge into the environment.

3 POULTRY PROCESSING PLANTS

The three poultry processing plants were selected for inclusion in this study because they were considered to be representative of large, well-operated Ontario plants, and because they employ a variety of wastewater treatment processes and effluent discharge options. No attempt was made to study operation and waste treatment processes; the descriptive material cited below was largely obtained from unpublished EPS data.

3.1 Plant A

The 1972 production at this plant was estimated at 49 million pounds (live weight) of chicken and turkey; in December, 1974, the plant processed about one million pounds of poultry per week. The plant effluent (process wastewater and domestic sewage) was estimated at approximately 2.2 million I gal per week. From the pump wet well, wastewater is pumped to an activated sludge treatment plant (Figure 1), after screening through Sweco screens, each with a capacity of 300 I gpm. The screened effluent flows to an aeration basin; the two 1,170,000 I gal basins are 16 feet deep and are equipped with 75 H.P. mechanized surface aerators. The secondary clarifier has a 40 ft. weir providing a design overflow rate of 4,260 I gpd per foot of weir length. Effluent from the secondary clarifier flows to a facultative lagoon which has a surface area of four acres and a depth of six feet. The maximum loading rate is reported to be 20 lb. BOD/acre/day. The lagoon effluent is chlorinated before discharge; a hypochlorite solution is used, with a dosage of 12 mg per l (6 pounds per day of chlorine). On May 27, 1975, the receiving ditch was virtually dry upstream from the effluent outfall, and the total ditch flow was derived from the plant effluent discharge.

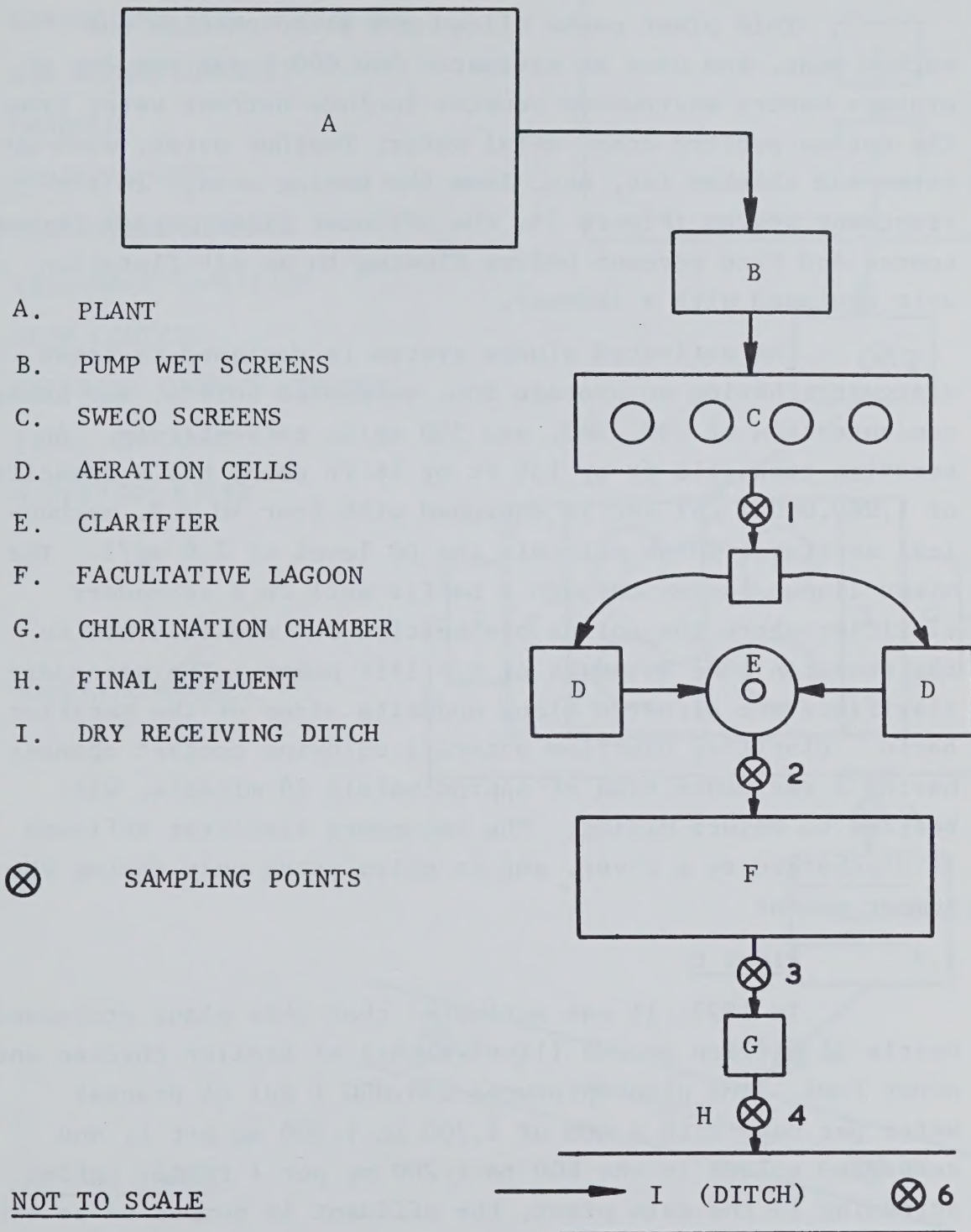


FIGURE 1. SCHEMATIC DIAGRAM, WASTE TREATMENT SYSTEM, PLANT A

3.2 Plant B

This plant packs sliced and diced chicken and turkey meat, and uses an estimated 540,000 I gal per day of process water; wastewater sources include defrost water from the cut-up poultry area, offal water, feather water, wash-up water and chicken fat, etc. from the boning area. In the treatment system (Figure 2), the effluent first passes through coarse and fine screens before flowing to an air flotation unit equipped with a skimmer.

The activated sludge system is designed to treat wastewater having an average BOD, suspended solids, and grease concentration of 790, 360, and 350 mg/l, respectively. An aeration tank (118 ft by 158 ft by 16 ft deep) has a capacity of 1,080,000 I gal and is equipped with four 40 H.P. mechanical aerators, which maintain the DO level at 2.0 mg/l. The mixed liquor passes through a baffle wall to a secondary clarifier where the solids are settled out and returned to the aeration zone by means of air lift pumps. The secondary clarifiers are situated along opposite sides of the aeration basin. Clarifier overflow enters a chlorine contact chamber having a residence time of approximately 30 minutes, with baffles to ensure mixing. The secondary clarifier effluent is discharged to a river, and is chlorinated only during the summer months.

3.3 Plant C

In 1972, it was estimated that this plant processed nearly 31 million pounds (live weight) of broiler chicken and other fowl. The plant produces 250,000 I gal of process water per day, with a BOD of 1,200 to 1,500 mg per l, and suspended solids in the 800 to 1,200 mg per l range. After screening in the main plant, the effluent is pumped to an air flotation unit equipped with a mechanical skimmer, where about one ton of grease per day is removed (Figure 3). The

- A. COARSE AND FINE SCREENING
- B. AIR FLOTATION UNIT
- C. MANHOLE
- D. AERATION CELL
- E. MECHANICAL AERATOR
- F. SECONDARY CLARIFIER
- G. WEIR CONTROL
- H. CHLORINE CONTACT CHAMBER
- I. FINAL EFFLUENT
- J. RECEIVING RIVER

⊗ SAMPLING POINTS

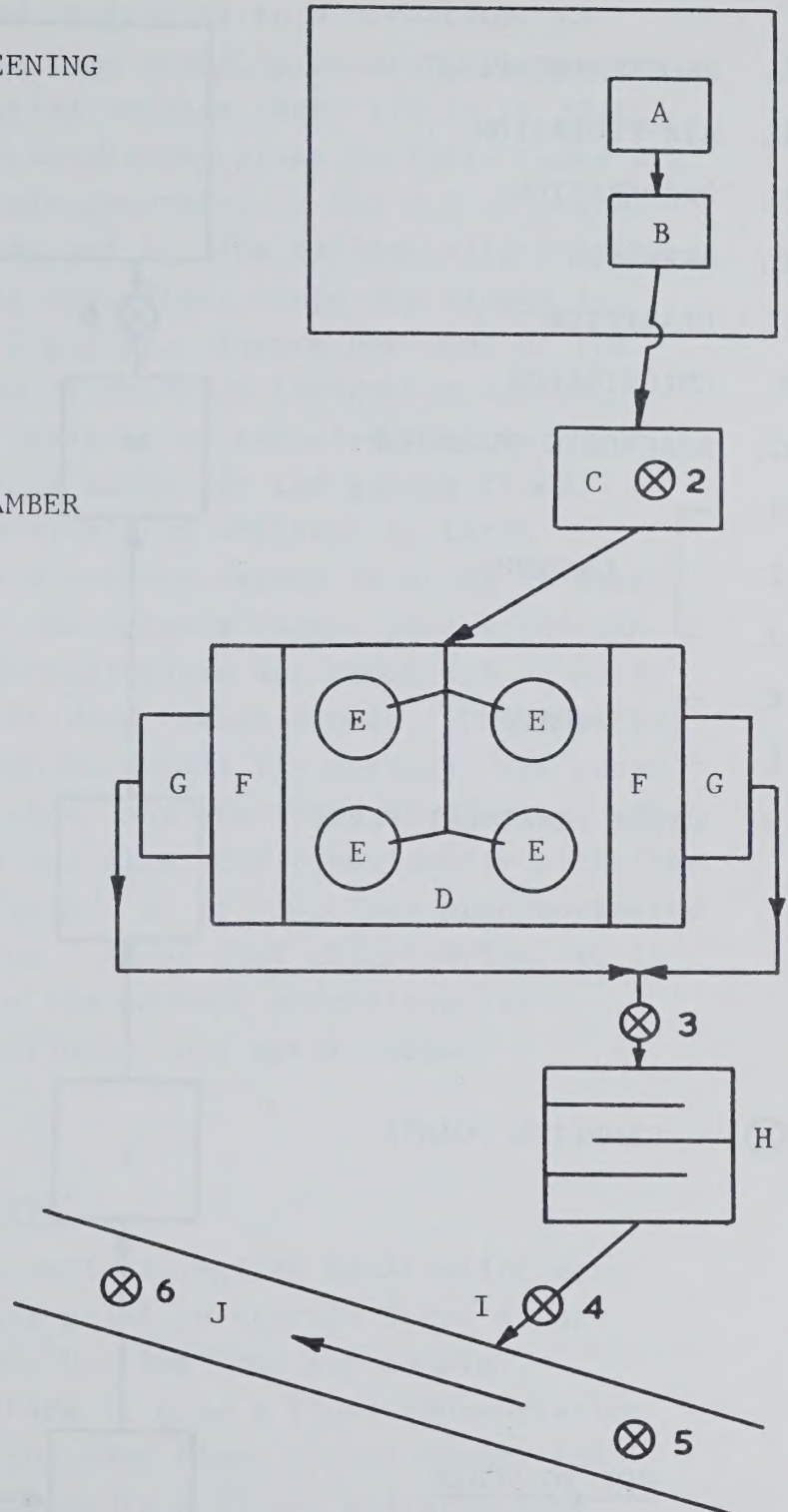


FIGURE 2. SCHEMATIC DIAGRAM, WASTE TREATMENT SYSTEM, PLANT B

- A. PROCESSING PLANT
- B. AIR FLOTATION
- C. PREPARATION
- D. AERATION
- E. CLARIFIER
- F. CHLORINATOR
- G. ANAEROBIC DIGESTER
- H.]
- I.] LAGOONS
- J.]
- K.]
- L.] PONDS
- M. WATER TREATMENT PLANT

⊗ SAMPLING POINTS

NOT TO SCALE

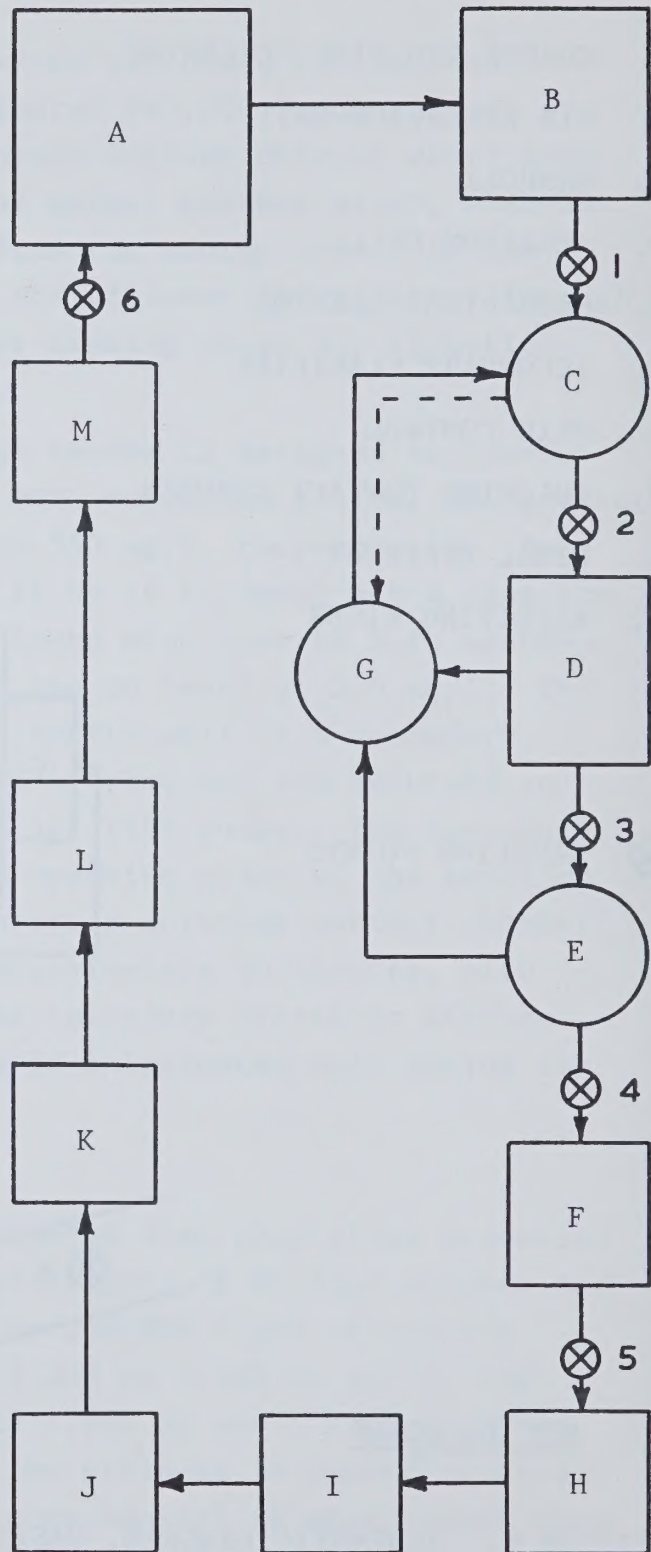


FIGURE 3. SCHEMATIC DIAGRAM, WASTE TREATMENT SYSTEM, PLANT C

"degreased" effluent is fed by gravity to a preaerator (a converted clarifier), where some settling occurs. The supernatant flows into two parallel aerator tanks (70 ft by 15 ft by 14 ft deep). Submerged open-ended pipes in these tanks are used to supply compressed air generated by 260 H.P. blowers, to maintain the DO at 2.0 mg per l. The biologically-treated effluent overflows into the clarifier, where the sludge is drawn off to an anaerobic digester. Twenty per cent of the digested sludge is disposed of by spray irrigation during summer and fall, and the remainder is recycled via the preaerator. Make-up water is added, in the spring flood period, from a creek. The clarified effluent is batch chlorinated, and pumped to a primary lagoon (6 acres in area; 5 ft deep). Effluent from the primary lagoon passes through two secondary lagoons in series; these are about 2.5 acres in area, and are 4.5 and 4.0 ft deep, respectively. The treated effluent then passes through two ponds (in series); the ponds are each about 5 acres in area, and are about 15 ft deep. The water is treated with clay and alum, and occasionally with lime during periods of algal blooms. It is sometimes prechlorinated before rapid sand filtration. After post chlorination, it is used as the water supply in the poultry processing plant. There is no discharge of effluent to a watercourse.

4 METHODS

4.1 Sampling Procedures

Grab samples for bacteriological examination were collected from each sampling point in sterile Pyrex glass-stoppered four-liter reagent bottles (two per sample), containing sodium thiosulphate to give a final concentration of 100 mg per l (1). At the same time, a grab sample for chemical analysis was collected in a 32 oz. polypropylene bottle.

Moore Swabs, as modified by Spino (35) and Dutka and Bell (11), were installed in the waste streams investigated, for very brief (3 to 4 hr) periods on the day of sample collection. Each swab was mounted on a 12"x12" piece of chicken-wire mesh. Six 1"x9" gauze strips, each made up of six layers of gauze, were gathered together at one end and tied with string to the wire mesh. The other ends of the gauze strips were spread, and separately tied to the mesh. The modified Moore Swab was wrapped in kraft paper and autoclaved before use. After exposure, the Swabs were retrieved from the sampling locations, inserted in sterile plastic bags, and iced, with the grab samples, in cooler chests (Thermos), for immediate delivery by automobile to the Laboratory. Salmonella isolation tests were set up on the day of sample collection; sample remainders were refrigerated overnight for examination for other parameters on the following morning.

4.2 Bacterial Pollution Index Parameter Tests

All effluent and water samples were subjected to APHA Standard Methods (1) Membrane Filter (MF) procedures for the estimation of coliform, fecal coliform and fecal streptococcus densities.

Coliforms: m-Endo Agar LES*
(35°C, 20 ± 2 hrs);

Fecal Coliforms: m-FC Agar* (water bath;
44.5°C, 20 ± 2 hrs);

Fecal Streptococci: m-Enterococcus Agar*
(35°C, 48 hrs).

The numbers of typical colonies appearing on the membranes for appropriate sample volumes were counted, and

* Unless otherwise specified, all test media used were Bacto Brand supplied by Difco Laboratories, Detroit

bacterial densities were calculated and recorded in terms of MF counts per 100 ml of sample. The membranes used were Gelman Metrical (GN-6), 47 mm diameter, 0.45 μ white gridded filters.

APHA Standard Methods (1) were also used in the determination of Standard Plate Counts; SPCs were calculated from the average counts for duplicate plates from the most appropriate decimal dilution (Plate Count Agar). Incubation was at 35°C for 24 and 48 hrs, and at 20°C for 3 and 5 days.

Ps. aeruginosa densities were estimated by means of an experimental tube-dilution (Most Probable Number) test assembled in this Laboratory. Confirmed MPNs of Ps. aeruginosa were determined by inoculation of Drakes 10 Presumptive Test Medium, with five tubes in each of three or more consecutive decimal dilutions (usually 100 or 10 to 10^{-4} ml), incubation at 38°C (water bath) for 4 days, and confirmation of all positive (fluorescent and/or pigmented) cultures on Acetamide Agar slants (41.5°C water bath incubation for 48 hrs). Each 100 ml presumptive test aliquot of sample was filtered through a sterile 0.45 μ MF (Gelman Metrical GN-6); the inoculated membrane was then transferred aseptically to the presumptive broth tube. All Acetamide-positive cultures were further confirmed by incubation on Kings B Agar slants for four days at 37°C; cultures with typical, green pigmentation and/or typical fluorescence under long-wave UV (Chromato-vue Model CC-20, Ultra-Violet Products, Inc.), were considered as Ps. aeruginosa.

Samples from Plant C only were also subjected to a parallel MF test for Ps. aeruginosa, as described by Levin and Cabelli (24), using m-PA Agar, with incubation for 48 hrs in sealed plastic bags in a water-jacketed air incubator controlled at 41.5°C. Only yellow and colourless colonies were excluded from the counts; all other (pink, brown, greenish) colonies were confirmed by transfer to Skim Milk Agar. Casein hydrolysis

after 24 hrs at 37°C was interpreted as evidence of the presence of Ps. aeruginosa; confirmed counts were recorded in terms of Ps. aeruginosa per 100 ml of sample.

A non-standard experimental Most Probable Number test for the Proteus-Providencia group was applied to all samples. The presumptive test medium was GN Broth (Hajna), with five tubes in each of three or more consecutive decimal dilutions (usually 10 to 10⁻⁴ ml), incubation at 37°C for two days, and confirmation of all tubes showing growth by transfer to Phenylalanine Agar slants for 24 hr incubation at 37°C. Positivity (phenylalanine deaminase activity) was determined by the addition of ferric chloride reagent to the slant (13). Results were expressed as the MPN of Proteus-Providencia group bacteria per 100 ml of sample. No claims can be made for the specificity of this tentative procedure; it does, however, appear to provide a reasonable estimate of Proteus numbers.

4.3 Salmonella Culturing Methods

The six Moore Swab strips were aseptically separated with sterile scissors and forceps. Two strips were transferred to separate 150 ml-capacity Fleakers (Kimble) containing 100 ml of sterile Tetrathionate Enrichment Broth; two strips were similarly enriched in Selenite Cystine Broth; and two strips were placed in separate Fleakers of Dulcitol Selenite Enrichment Broth. One Fleaker of each enrichment broth was incubated at 37°C, while the duplicate Fleaker of each broth was incubated at 41.5°C in a water bath.

All enrichment broths were incubated for 24 and 48 hrs before plating (one loopful of broth) on each of three selective agars: Brilliant Green (BG) Agar; Xylose Lysine Dulcitol (XLD) Agar (Taylor), and Bismuth Sulphite (BiS) Agar (Wilson and Blair). Each plate was incubated for 24 hrs at the incubation temperature used for enrichment (37° and 41.5°C), before examination and colony selection.

Similarly, six aliquots of each grab sample were filtered to provide inocula for Salmonella enrichment; aliquots of 500 ml were used in the case of raw and partially treated wastes, while one l aliquots of treated wastes and water were examined. Each aliquot was filtered separately through 47 mm glass-fiber prefilters (Sartorius, No. 13400); usually, 5 to 7 prefilters were required for each aliquot filtration, depending on sample turbidity. The filtrates were retained in sterile filter flasks, and were refiltered through sterile 0.45 μ membrane filters (Gelman GN-6); normally, 5 to 7 membrane filters were required for each aliquot filtration. All pre-filters and MFs from each aliquot filtration were transferred to a Fleaker containing an enrichment broth. The array of media, and incubation temperatures and times, was identical to those cited for the Moore Swab technique outlined above.

The described test battery, while unwieldy, permitted the concurrent evaluation of a total of 36 "isolation pathways"; for each sample, the test battery included:

- three enrichment broths x 2 temperatures;
- two incubation periods (x2); and
- three selective plating agars (x3).

After 24 hr incubation, each agar plate was carefully examined for the presence of Salmonella "suspicious" colonies. If any typical, or atypical but "possible", colonies were found, representative colonies (1 to 7) were transferred to Tryptone Glucose Extract (TGE) Agar slants for holding purposes. All cultures were later restreaked on MacConkey Agar purification plates. Typical and atypical non-lactose-fermenters were reisolated in pure culture to TGE Agar slants, and subsequently were subjected to cytochrome oxidase, Triple Sugar Iron (TSI) Agar (acid, gas and H₂S), and phenylalanine deaminase tests, all with incubation of 37°C. Those cultures still considered on the basis of screening test results as possible salmonellae

were subjected to a battery of 21 biochemical tests, using the Minitek (BBL) disc system. Tests included were: ONPG, phenylalanine deaminase, urea, H₂S, lysine decarboxylase, ornithine decarboxylase, arginine dehydrolase, nitrate, indol, Voges-Proskauer, citrate, and the following sugar fermentation tests, arabinose, dulcitol, glucose, malonate, raffinose, rhamnose, salicin, sorbitol, sucrose and xylose.

Cultures biochemically identified as salmonellae were further identified serologically according to the Kauffman-White schema and the methods of Edwards and Ewing (12). Slide agglutination tests with polyvalent O antisera and specific single factor antisera (Difco) were conducted to identify somatic antigens. Flagellar (H) antigens were determined using Spicer Edwards (12) antisera (Difco). Representative cultures of each serotype found were submitted to the National Enteric Reference Centre, Laboratory Centre for Disease Control, Department of National Health and Welfare, Ottawa, for final identification and confirmation.

4.4 Fecal Coliform Isolate Identification

All, or representative, typical fecal coliform colonies appearing on m-FC Agar MF preparations for appropriate dilutions of each sample were isolated for pure culture study. The isolates were restreaked on Levine Eosin Methylene Blue (EMB) Agar purification plates. Pure cultures isolated from these plates were subjected to the following biochemical identification tests: lactose fermentation, with production of acid and gas, at 35° and 44.5°C; cytochrome oxidase; indol; methyl red; Voges-Proskauer; citrate (Simmons); ornithine decarboxylase; and motility (plate test, semi-solid agar, 35°C).

4.5 Antibiotic Resistance Tests

All pure culture isolates of salmonellae and fecal coliforms were tested for resistance to antibiotic drugs. Nutrient Broth cultures of the test organisms were grown

overnight at 35°C; 1 : 10 dilutions of the broth cultures in standard phosphate buffer (3) were poured on the surface of sterile plates of Diagnostic Sensitivity Test (DST) Agar (Oxoid; Med-Ox Chemicals Ltd., Ottawa), and the excess discarded (to produce a lawn culture). After the plates were air-dried, high-potency Multodisks (Oxoid Codes 4219S and 4220S; Med-Ox Chemicals Ltd.) were placed on the agar surfaces for overnight incubation at 35°C. Diameters (mm) of the zones of growth inhibition around each disk were measured to determine the antibiotic susceptibility of the test cultures. The twelve antibiotics employed are listed, with their abbreviations, concentrations, and zone-size categories, in Table 1.

4.6 Methods of Chemical Analysis

Grab samples of wastewater and water from the same sampling points were analysed for the following parameters: Chemical Oxygen Demand (COD), Suspended Solids (SS), Volatile Suspended Solids (VSS), and Total Dissolved Solids (TDS), using APHA Standard Methods (1); tests were conducted for Total Phosphate (TP), Total Kjeldahl Nitrogen (TKN), Nitrates (NO₃) and Nitrites (NO₂), using automated methods (Technicon), and for Total Organic Carbon (TOC) using a Beckman Carbonaceous Analyser. Results for all parameters were expressed as mg per 1 of sample.

5 RESULTS AND DISCUSSION

5.1 Bacterial Pollution Indices

MF counts for the standard bacterial public health parameters (coliforms, fecal coliforms and fecal streptococci), for each of the three plants studied, are recorded in Tables 2, 3 and 4. Similarly, Standard Plate Counts and Ps. aeruginosa and Proteus-Providencia density estimates are reported in Tables

TABLE 1 . ANTIBIOTIC SENSITIVITY ZONE SIZE CATEGORIES

Antibiotic		Zone Size in mm		
		Resistant (or less)	Intermediate	Susceptible (or more)
TE 50	Tetracycline	14	15-18	19
PN 25	Ampicillin	11	12-13	14
KF 25	Cephalothin	14	15-17	18
CN 10	Gentamycin	12	13-14	15
K 30	Kanamycin	13	14-17	18
S 25	Streptomycin	11	12-14	15
F 200	Nitrofurantoin	14	15-18	19
PY100	Carbenicillin	17	18-22	23
PB300	Polymyxin B	10	11-14	15
CT 10	Colistin Sulfate	9	10-13	14
E 50	Erythromycin	13	14-17	18
G 300	Sulphafurazole	12	13-16	17

TABLE 2 . BACTERIAL MF COUNTS, PLANT A, 1975

Sta. No.	Sample Source	MF Count per 100 ml.		
		Coliform	Fecal Coliform	Fecal Strep.
1	Screened Effluent	6,300,000	1,700,000	1,700,000
2	Treated Effluent	130,000	97,000	18,000
3	Lagoon Effluent Before Cl ₂	5,300	4,800	2,700
4	Lagoon Effluent After Cl ₂ (2.3 mg/l)	440	<2	20
*6	Receiving Stream, Downstream (Cl ₂ 1.6 mg/l)	380	8	16

* There was no flow in the receiving ditch upstream from the effluent outfall (Planned Station 5).

5, 6, and 7. Miscellaneous on-site data (temperatures, DO and Cl₂ concentrations) at the various wastewater sampling points are cited in Table 8.

Counts for the three standard parameters, recorded for raw screened wastes at the three plants, were comparable to those usually associated with raw sewage, which typically contains coliform bacteria in the 10⁶ to 10⁹ per 100 ml range, with lesser numbers of fecal coliforms and fecal streptococci. In Plants A and B, activated sludge treatment effected very significant reductions (93 to 99.9 per cent) in counts for the three standard pollution indices, for both Standard Plate Counts, and for Ps. aeruginosa numbers. Chlorination disinfection caused a further major reduction (90 per cent or

TABLE 3 . BACTERIAL MF COUNTS, PLANT B, 1975

Sta. No.	Sample Source	MF Count per 100 ml.		
		Coliform	Fecal Coliform	Fecal Strep.
2	Raw Influent, After Air Flotation	2,900,000	720,000	1,600,000
3	Treated Effluent Before Cl ₂	100,000	54,000	18,000
4	Chlorinated Effluent (0.1 mg/l Cl ₂)	340	6	80
5	Receiving Stream (Upstream from outfall)	8,100	610	450
6	Receiving Stream (Downstream from outfall)	5,500	760	290

more) in bacterial numbers.

Conversely, aeration treatment of wastes in Plant C resulted in multiplication of all bacterial parameters, with increases in numbers of at least one order of magnitude at some point in the system. Only minor net reductions in bacterial numbers resulted from settling (Sta. 4) and ineffective disinfection (Sta. 5). There was a net increase in fecal coliform densities during treatment. This was associated with fecal coliform : fecal streptococcus ratios of more than 4.0 at Stations 3 to 6, inclusive; such ratios are normally considered to be indicative of human wastes (15). It should be noted, however, that recent studies in this Laboratory have shown that there is a marked increase, usually greater than one

TABLE 4 . BACTERIAL MF COUNTS, PLANT C, 1975

Sta. No.	Sample Source	MF Count per 100 ml.		
		Coliform	Fecal Coliform	Fecal Strep.
1	Screened Effluent, After Air Flotation and Skimming	58,000,000	13,000,000	16,000,000
2	Effluent, After Pre-Aeration	200,000,000	130,000,000	38,000,000
3	Effluent, from Aerator	100,000,000	70,000,000	10,000,000
4	Treated Effluent, After Settling	38,000,000	35,000,000	1,800,000
5	Treated Effluent, After Cl ₂ (<0.1 mg/l)	37,000,000	35,000,000	2,100,000
6	Treated Water, Plant Input	90	36	4

order of magnitude, in all index bacterial counts in domestic and wild animal and bird feces during the immediate post-excretion period, and that the FC : FS ratio was frequently greater than 4.0 during this period (42). Only after extended storage of feces did there occur a reversion of the FC : FS ratios to less than 0.7, normally regarded as characteristic of animal wastes (15). Further waste treatment in a series of lagoons and ponds was not monitored. Plant C reclaimed input water (Sta. 6) after sand filtration and chlorination had reasonably low bacterial counts, but could not be considered as potable.

TABLE 5 . BACTERIAL DENSITY DATA, PLANT A, 1975

Sta. No.	Sample Source	Standard Plate Count per ml.					MPN per 100 ml.	
		35° 24 hrs.	35° 48 hrs.	20° 3 days	20° 5 days		<u>Pseudomonas aeruginosa</u>	<u>Proteus- Providencia</u>
1	Screened Effluent	550,000	780,000	1,700,000	1,900,000		4,900	130,000
2	Treated Effluent	10,000	18,000	20,000	46,000		170	2,600
3	Lagoon Effluent Before Cl ₂	38,000	49,000	42,000	50,000		22	1,600,000
4	Lagoon Effluent After Cl ₂ (2.3 mg/l)	150	170	340	4,200		<2	33
*6	Receiving Stream, Downstream (Cl ₂ 1.6 mg/l)	670	1,400	4,500	4,500		<2	170

* There was no flow in the receiving ditch upstream from the effluent outfall (Planned Station 5)

TABLE 6 . BACTERIAL DENSITY DATA, PLANT B, 1975

Sta. No.	Sample Source	Standard Plate Count per ml.					MPN per 100 ml.	
		35° 24 hrs.	35° 48 hrs.	20° 3 days	20° 5 days		<u>Pseudomonas aeruginosa</u>	<u>Proteus- Providencia</u>
2	Raw Influent, After Air Flotation	4,900,000	6,800,000	9,000,000	9,400,000		22,000	2
3	Treated Effluent Before Cl ₂	330,000	470,000	390,000	550,000		700	23,000
4	Chlorinated Effluent (0.1 mg/l Cl ₂)	6,300	14,000	18,000	27,000		21	460
5	Receiving Stream (Upstream from outfall)	1,600	3,900	7,300	7,600		8	13,000
6	Receiving Stream (Downstream from outfall)	1,500	2,800	2,000	2,300		11	49,000

TABLE 7 . BACTERIAL DENSITY DATA, PLANT C, 1975

Sta. No.	Sample Source	Standard Plate Count per ml.				MPN per 100 ml.	
		35° 24 hrs.	35° 48 hrs.	20° 3 days	20° 5 days	<u>Pseudomonas aeruginosa</u>	<u>Proteus- Providencia</u>
1	Screened Effluent, After Air Flotation and Skimming	6,500,000	6,900,000	8,300,000	9,100,000	21,000	17,000,000
2	Effluent, After Pre-Aeration	37,000,000	37,000,000	40,000,000	41,000,000	33,000	>160,000,000
3	Effluent, from Aerator	99,000,000	100,000,000	160,000,000	170,000,000	79,000	>160,000,000
4	Treated Effluent, After Settling	70,000,000	70,000,000	88,000,000	88,000,000	22,000	24,000,000
5	Treated Effluent, After Cl ₂ (<0.1 mg/l)	95,000,000	95,000,000	120,000,000	120,000,000	110,000	2,800,000
6	Treated Water, (Plant Input)	2	2	2	2	<2	<2

TABLE 8 . MOORE SWAB EXPOSURE TIMES, TEMPERATURE, DISSOLVED OXYGEN, AND CHLORINE CONCENTRATION, AT TIME OF SAMPLE COLLECTION, PLANTS A, B AND C

Sta. No.	Moore Swab Exposure hrs : min	Temp. °C	O ₂ mg/l	Cl ₂ mg/l
<u>Plant A</u>				
1	3 : 10	15.0	9.4	No Test
2	3 : 30	18.0	0.8* 4.1**	No Test
3	3 : 20	21.5	0.6* 5.5**	No Test
4	3 : 30	21.0	6.2* 7.6**	2.3
6	No Test	21.0	7.6	1.6
<u>Plant B</u>				
2	3 : 35	18.0	0.2	No Test
3	3 : 50	21.0	5.1	No Test
4	3 : 55	21.0	4.8* 7.2**	0.1
5	3 : 55	21.5	7.6	<0.1
6	3 : 55	22.0	7.5	<0.1
<u>Plant C</u>				
1	3 : 45	22.0	6.8	<0.1
2	3 : 40	23.0	0.6	<0.1
3	3 : 40	23.5	0.8	0.3
4	3 : 40	23.0	0.8	<0.1
5	3 : 40	23.0	2.8	<0.1
6	No Test	No Test	No Test	1.2

* DO Before Weir Overflow

** DO After Weir Overflow

As expected, Standard Plate Count densities (35° and 20°C) applied to Plant A and B samples, tended to increase with extended incubation. This, however was not true for Plant C wastes; evidently aerobic heterotrophs, presented with near-optimum nutrient and temperature conditions, produced a maximum number of colonies in the shorter, standard incubation period.

Ps. aeruginosa densities declined to low levels during treatment in Plants A and B, but multiplied in Plant C wastes undergoing aeration treatment at 22° - 23°C. The MF method of Levin and Cabelli (24), when applied in parallel to the MPN test for the estimation of Ps. aeruginosa numbers in Plant C samples, gave significantly lower density estimates:

Sta. No.	<u>Ps. aeruginosa</u> /100 ml.	
	<u>MPN</u>	<u>MF</u>
1	21,000	16,000
2	33,000	20,000
3	79,000	39,000
4	22,000	13,000
5	110,000	11,000
6	<2	0

The tribe Proteeae consists of two genera, Proteus and the much less ubiquitous Providencia. Proteus organisms are frequently found in the feces of humans undergoing oral antibiotic therapy, and their presence tends to complicate the search for salmonellae. While the two genera are not considered to be highly pathogenic, they have both been incriminated in cases of gastroenteritis, sporadic and epidemic, and they frequently are responsible for infections of the urinary tract. Data presented herein provide presumptive evidence that Proteus was capable of proliferation under treatment

conditions (Plants A and B, Stations 3), and that these bacteria also proliferated throughout the part of the Plant C treatment system studied. While there is no direct evidence that other potentially pathogenic Enterobacteriaceae, including salmonellae, also multiplied in these wastes, the possibility that they could do so appears to exist.

5.2 Chemical Pollution Indices

Chemical analytical data obtained for samples from the wastewater treatment systems of the three poultry processing plants are reported in Tables 9, 10 and 11.

Waste treatment at all three plants operated efficiently to remove COD, organic carbon and suspended solids. In terms of treatment efficiency achieved in small domestic sewage treatment plants, the reduction of COD by more than 90 per cent could be considered as good, as was the 82 - 98 per cent removal of SS.

Total organic (Kjeldahl) nitrogen values in the raw wastes varied from 60 to 116 mg per l; these were reduced during treatment to values between 1 and 24 mg per l. At the same time, in Plant A, the nitrate-nitrogen value rose after aeration to 3.5 mg per l, and declined to 0.5 mg per l after lagooning; the values in Plant B rose after aeration from 4.0 to 31.2 mg per l. It is a normal pattern in waste treatment that nitrate-nitrogen increases with oxidation of organic-nitrogenous material. In Plant C, the nitrate level was highest at Station 1, and declined thereafter, except for a slight rise after aeration. Final nitrate values were all satisfactorily low.

Phosphate values, initially between 8 and 17 mg per l, declined during treatment by about the amount expected in biological treatment systems without phosphate removal. The values of 6 mg per l in the final effluents of Plants A and B

TABLE 9 . CHEMICAL ANALYTICAL DATA, PLANT A, 1975

Sta. No.	Sample Source	C.O.D.	T.O.C.	T.K. (N)	T.P.	S.S.	V.S.S.	T.D.S.	NO ₃	NO ₂
1	Screened Effluent	928	500	88	8.7	276	260	262	.05	N.D.
2	Treated Effluent	67	24	9.0	5.2	47	44	342	3.5	2.0
3	Lagoon Effluent, Before Cl ₂	72	32	15	6.7	49	47	426	0.5	.07
4	Lagoon Effluent, After Cl ₂	79	29	14.5	6.0	25	22	408	0.20	.06
6	Receiving Stream, Downstream	94	34	14.5	6.0	51	34	456	0.21	.07

N.D. = None Detected. Results for all parameters reported as mg per liter.

TABLE 10. CHEMICAL ANALYTICAL DATA, PLANT B, 1975

Sta. No.	Sample Source	C.O.D.	T.K. (N)	T.P.	S.S.	V.S.S.	D.S.	V.D.S.	NO ₃	NO ₂	T.O.C.
2	Raw Influent, After Air Flotation	1886	60	9.2	500	204	600	292	4.0	N.D.	680
3	Treated Effluent Before Cl ₂	33	2	5.8	23	15	786	247	31.2	.16	12
4	Chlorinated Effluent (0.1 mg/l Cl ₂)	35	1	6.0	18	14	792	222	29.0	.05	13
5	Receiving Stream (Upstream from outfall)	15	0.5	0.1	24	12	352	123	2.0	.03	7
6	Receiving Stream (Downstream from outfall)	16	0.5	0.1	18	9	353	113	0.3	.03	7

N.D. = None detected . Results for all parameters reported as mg per liter.

TABLE 11. CHEMICAL ANALYTICAL DATA, PLANT C, 1975

Sta. No.	Sample Source	C.O.D.	T.K. (N)	T.P.	S.S.	V.S.S.	D.S.	V.D.S.	NO ₃	NO ₂	T.O.C.
1	Screened Effluent, After Air Flotation and Skimming	2714	116	16.8	950	900	906	294	11.3	.044	1000
2	Effluent, After Pre-Aeration	2268	102	15.9	804	724	544	150	2.9	.019	950
3	Effluent, From Aerator	3369	75	14.5	2010	1830	456	118	4.7	.008	52
4	Treated Effluent, After Settling	124	24	2.9	25	24	485	116	1.4	.062	29
5	Treated Effluent, After Cl ₂ (<0.1 mg/l)	134	23.8	3.0	76	58	446	86	1.0	.060	38
6	Treated Water (Plant Input)	3.8	1.0	0.1	4	3	336	89	0.5	.011	10

Results for all parameters reported as mg per liter.

do not meet the objectives of 1 mg per l P applied to municipal treatment plants.

Chlorination had no significant effect on values recorded for chemical parameters, with the exception of suspended solids; SS values were lower after chlorination in Plant A and B effluents, but higher at Plant C. These may reflect physical effects (settling in the first two cases, and agitation at Plant C).

5.3 Fecal Coliform Isolate Identification

Data obtained from the biochemical identification of 322 representative fecal coliform cultures isolated from wastewater samples from the previously described stations are cited in Tables 12, 13 and 14, and are summarized, for the three Plants, in Table 15.

Escherichia coli biotypes accounted for 311 of the 322 fecal coliform strains isolated. Thus, for this study, the term, "fecal coliform" was virtually synonymous with "E. coli". There were nine Enterobacter aerogenes isolates, and only two Klebsiella strains were found.

Of the 311 E. coli isolates, 286 (92 per cent) grew with typical metallic sheen on Levine EMB Agar purity plates. Only one of eleven isolates of other biotypes had colonies with similar sheen. Of the 300 E. coli type 1 strains, only 167 were completely typical (44.5°C gas-positive; ornithine decarboxylase positive; motile). There were 90 non-motile cultures, 41 ornithine negative isolates, and four strains which failed to produce gas from EC Medium within 48 hours at 44.5°C. Six of nine E. aerogenes isolates were also anaerogenic in the EC test. Thus ten of the 322 isolates could technically be regarded as EC anaerogenic false positives; this is, however, a rather typical finding, because EC aerogenicity is

TABLE 12. IDENTIFICATION OF FECAL COLIFORM ISOLATES, PLANT A, 1975

Biotype and IMViC	FC (Gas) 45.5°C	Ornith. Decarb.	Motility 35°C	Fecal Coliform Isolates, Station No.					
				1	2	3	6	No. (%)	
<u>Escherichia coli</u>				10	18	16	7	51 17 6 2	(64.6) (21.5) (7.6) (2.5)
+	+	+	+	6	5	6			
+	+	+	+		4	2			
+	+	-	+			2			
+	-	-	+						
<u>Enterobacter aerogenes</u>				2				2	(2.5)
-	-	+	+						
<u>Klebsiella</u>				1				1	(1.3)
-	+	-	-						
-	+	-	-						
Totals				19	27	26	7	79	

TABLE 13. IDENTIFICATION OF FECAL COLIFORM ISOLATES, PLANT B, 1975

Biotype and IMViC	FC (Gas) 45.5°C	Ornith. Decarb.	Motility 35°C	Fecal Coliform Isolates, Station No.						No. (%)
				2	3	4	5	6		
<u>Escherichia coli</u>										
+	+	+	+	15	15	5	24	20	79	(73.1)
+	+	+	-	6	2		1	3	12	(11.1)
+	+	-	+	2	6			1	9	(8.3)
-	+	-	+	2					2	(1.9)
<u>Enterobacter aerogenes</u>										
-	+	+	+		1				1	(0.9)
-	+	-	+		1	1			2	(1.9)
-	-	+	+		1				1	(0.9)
-	-	+	+	1					1	(0.9)
<u>Klebsiella</u>										
-	+	-	-					1	1	(0.9)
Totals				26	26	6	25	25	108	

TABLE 14. IDENTIFICATION OF FECAL COLIFORM ISOLATES, PLANT C, 1975

Biotype and IMViC	EC (Gas) 45.5°C	Ornith. Decarb.	Motility 35°C	Fecal Coliform Isolates, Station No.						No.	(%)
				1	2	3	4	5	6		
<u>Escherichia coli</u>											
+	+	+	+	4	8	7	7	6	5	37	(27.4)
+	+	+	-	8	10	13	13	10	6	60	(44.4)
+	+	+	-		1					1	(0.7)
+	+	+	+			1				1	(0.7)
+	+	-	+	1	6	4	3	6	5	25	(18.5)
-	+	+	+	1			3			3	(2.2)
-	+	+	-		1				2	3	(2.2)
-	+	-	+			1		2		3	(2.2)
<u>Enterobacter aerogenes</u>											
+	-	-	+					1		1	(0.7)
-	-	+	-			1				1	(0.7)
Totals				14	26	26	26	25	18	135	

TABLE 15. IDENTIFICATION OF FECAL COLIFORM ISOLATES, 1975

Biotype and IMViC				Fecal Coliform Isolates, Plant			
EC (Gas) 45.5°C				No. (%)			
Ornith. Decarb.				A B C			
Motility 35°C				A B C			
<u>Escherichia coli</u>							
+	-	+	+	51	79	37	167 (51.9)
+	-	+	+	17	12	60	89 (27.6)
+	-	+	+	6	9	25	40 (12.4)
+	-	+	+			1	1 (0.3)
+	-	+	+			1	1 (0.3)
+	-	+	+	2			2 (0.6)
-	-	+	+			3	3 (0.9)
-	-	+	+		2	3	3 (0.9)
-	-	+	+			3	5 (1.6)
<u>Enterobacter aerogenes</u>							
-	+	+	+		1		1 (0.3)
-	+	+	+		2		2 (0.6)
-	+	+	+	2	1		3 (0.9)
-	-	+	+		1	1	1 (0.3)
-	-	+	+				1 (0.3)
+	+	+	+			1	1 (0.3)
<u>Klebsiella</u>							
-	-	+	+	1	1		2 (0.6)
Totals				79	108	135	322

not a requirement for MF fecal coliform test positivity.

In general, the pure culture isolate data confirm the validity and specificity of the fecal coliform MF test as applied in this study.

5.4 Isolation of Salmonellae

In an attempt to isolate all salmonellae capable of growth on the selective plating agars used, one or more colonies representing all "possible" Salmonella colonies appearing on the plates were isolated for pure culture study. A total of 1,774 cultures were isolated in this way. It should be noted that many selected colonies were morphologically atypical; many were considered as marginal choices, and would probably have been ignored in a routine survey. Nevertheless, occasional very-atypical colonies confirmed as salmonellae. Under the terms of the selection procedure, it is not surprising that only 534 (30.1 per cent) of the cultures were confirmed, biochemically and serologically, as salmonellae.

As shown in Table 16, cultures were readily eliminated as lactose-fermenters on MacConkeys Agar purity plates, and as green-pigmented pseudomonads and other cytochrome oxidase-positive bacteria; the frequent interference by pseudomonads in enrichment cultures is well known. In addition, the phenylalanine deaminase reaction was valuable in eliminating the large number of Proteus - Providencia group strains included. Once these screening tests were completed, a relatively small number of non-salmonellae were eliminated on the basis of TSI Agar reactions and a comprehensive battery of biochemical tests (Minitex) of which the ONPG test was of prime importance.

As cited in Table 17, salmonellae were isolated from waste samples associated with all three poultry processing plants studied. Salmonellae found are cited by serotype, antigen structure, and frequency of isolation, for each plant. The origin of these isolates, by Sampling Station, is shown for each plant in Tables 18, 19 and 20.

TABLE 16 . BIOCHEMICAL SCREENING OF SALMONELLA-SUSPICIOUS ISOLATES FROM SELECTIVE AGAR PLATES

Biochemical Screening Results, Salmonella-Suspicious Isolates	No. (%) of Isolates			
	Plant A	Plant B	Plant C	Totals
<u>No. of Samples</u>				
Moore Swabs	4	5	5	14
Filtered	5	5	6	16
Total No. of Isolates	391	674	709	1,774
<u>Isolates Eliminated by:</u>				
MacConkey Agar (Lactose +)	14 (3.6)	129 (19.1)	35 (4.9)	178 (10.0)
Green Pigmentation (Pseudomonads)	109 (27.9)	94 (13.9)	66 (9.3)	269 (15.2)
Cytochrome Oxidase +	42 (10.7)	173 (25.7)	118 (16.6)	333 (18.8)
Phenylalanine deaminase +	54 (13.8)	96 (14.2)	182 (25.7)	332 (18.7)
TSI Agar and Biochemical Reactions (Minitex)	25 (6.4)	41 (6.1)	62 (8.7)	128 (7.2)
Typical Salmonellae (Confirmed)	147 (37.6)	141 (20.9)	246 (34.7)	534 (30.1)

TABLE 17. SALMONELLA ISOLATES, BY SEROTYPE, ANTIGENIC STRUCTURE, AND FREQUENCY OF ISOLATION, PLANTS A, B, AND C, 1975

Plant	Salmonella Serotype	Antigenic Structure	Times Isolated
A	S. chester	4,12 : e,h : e,n,x	126
	S. saint paul	1,4,12 : e,h : 1,2	14
	S. montevideo	6,7 : g,m,s : -	6
	S. infantis	6,7 : r : 1,5	1
B	S. oranienberg	6,7 : m,t : -	73
	S. heidelberg	4,12 : r : 1,2	34
	S. bredeney	1,4,12 : 1,v : 1,7	20
	S. thompson	6,7 : k : 1,5	7
	S. saint paul	1,4,12 : e,h : 1,2	4
	S. infantis	6,7 : r : 1,5	3
C	S. manhattan	6,8 : d : 1,5	70
	S. schwarzengrund	1,4,12,27 : d : 1,7	160
	S. indiana	1,4,12 : z : 1,7	13
	S. heidelberg	4,12 : r : 1,2	2
	S. blockley	6,8 : k : 1,5	1

TABLE 18. SALMONELLA ISOLATES, PLANT A, 1975

Sta. No.	Sampling Station	Salmonella Serotype Found	Times Isolated	
			Moore Swab	Filtered Samples
1	Screened Effluent	S. chester	18	22
		S. saint paul	1	2
		S. montevideo	1	1
2	Treated Effluent	S. chester	14	29
		S. saint paul	0	4
		S. montevideo	0	4
		S. infantis	0	1
3	Lagoon Effluent Before Cl ₂	S. chester	18	25
		S. saint paul	2	5
4	Lagoon Effluent After Cl ₂ (2.3)	None		
6	Receiving Stream (dry ditch) 1.6 mg/1 Cl ₂	None	No Test	

TABLE 19. SALMONELLA ISOLATES, PLANT B, 1975

Sta. No.	Sampling Station	Salmonella Serotype Found	Times Isolated	
			Moore Swab	Filtered Samples
2	Raw Influent, After Air Flotation	S. oranienberg	20	3
		S. heidelberg	7	11
		S. bredeney	4	0
		S. saint paul	0	1
3	Treated Effluent, Before Cl ₂	S. oranienberg	2	17
		S. heidelberg	4	10
		S. bredeney	6	7
		S. saint paul	0	3
4	Chlorinated Effluent After Cl ₂ (0.1)	S. oranienberg	0	25
		S. heidelberg	0	2
		S. bredeney	0	3
5	Receiving Stream (Upstream)	S. oranienberg	0	6
		S. thompson	0	7
6	Receiving Stream (Downstream)	S. infantis	0	3

* Includes one rough strain, Salmonella OR: 1,2(I)

TABLE 20. SALMONELLA ISOLATES, PLANT C, 1975

Sta. No.	Sampling Station	Salmonella Serotype Found	Times Isolated	
			Moore Swab	Filtered Samples
1	Screened Effluent	S. schwarzengrund	20	21
		S. manhattan	10	6
		S. indiana	4	0
2	Effluent, After Pre- Aeration	S. schwarzengrund	17	27
		S. manhattan	2	5
		S. blockley	0	1
3	Effluent from Aerator	S. schwarzengrund	21	11
		S. manhattan	8	5
		S. indiana	0	1
		S. heidelberg	1	0
4	Treated Effluent After Settling	S. schwarzengrund	9	14
		S. manhattan	3	8
		S. indiana	0	3
		S. heidelberg	0	1
5	Treated Effluent After Cl ₂ (<0.1 mg/l)	S. schwarzengrund	9	11
		S. manhattan	8	15
		S. indiana	1	4
6	Treated Water (Plant Input)	None	No Test	

In Plant A wastes, S. chester was the predominant serotype, and was readily isolated from 500 ml filtered aliquots of raw and treated effluent. S. saint paul and S. montevideo were found less frequently in the same samples. A single culture of S. infantis, well known as a poultry pathogen, was isolated from treated effluent. Disinfection (2.3 mg per l of chlorine at the time of sample collection) was apparently effective in eliminating salmonellae from the lagoon effluent, as determined before and after effluent discharge to a dry ditch.

S. oranienberg was the dominant Plant B serotype, but S. heidelberg and S. bredeney were also readily isolated from 500 ml aliquots of raw and treated wastes. These three serotypes also were isolated from the inadequately disinfected (0.1 mg per ml chlorine) effluent as discharged to the receiving stream. One rough S. heidelberg strain isolated from the Moore Swab at Station 3 (Dulcitol Selenite Enrichment, 41.5°C, BiS Agar) was identified as Salmonella OR: 1,2 (I) by the Enteric Reference Centre. One liter samples of stream water collected upstream from the effluent outfall contained S. oranienberg, as well as S. thompson, a common serotype of avian origin. Since S. infantis was the only serotype isolated from samples collected at Station 6 in the receiving stream downstream from the Plant B effluent outfall, the single series of samples did not demonstrate that salmonellae from Plant B wastes were contributed to the receiving stream.

S. schwarzengrund and S. manhattan were the dominant salmonellae in wastes from Plant C, and were consistently present at Stations 1 to 5; S. indiana was found less frequently at all but Station 2. Two strains of S. heidelberg and one of S. blockley were also identified. No salmonellae were found in one l filtered samples of the treated plant input water (Station 6).

It is probable that Salmonella serotypes, found in raw and treated wastes from each of the three processing plants, originated from the particular poultry flocks processed immediately prior to sample collection. Thus the serotype incidence and distribution could be expected to vary in direct association with the carriage of particular serotypes contaminating flocks received at the plants. It is also evident that the waste treatment systems applied did not eliminate salmonellae introduced with raw wastes; effective disinfection before discharge will be necessary to produce Salmonella-free final effluents.

5.5 Antibiotic Resistance, Fecal Coliforms and Salmonellae

Antibiotic resistance data for the 322 fecal coliform isolates described in Section 5.3 are summarized in Table 21 for each of twelve antibiotic disk tests. Multiple resistance is summarized, numerically, in Table 22, and is shown by resistance pattern in Table 23. Antibiotic zone size category distribution is cited in Table 24, with comparable data for the 534 Salmonella isolates described in Section 5.4. Antibiotic resistance patterns determined for these salmonellae are given in Tables 25 and 26.

While the incidence of antibiotic resistance differed from plant to plant, clear trends emerged. Fecal coliforms tended to have a high incidence of resistance to some individual antibiotics, particularly to tetracycline (53.7 per cent) and streptomycin (62.1 per cent), and a high frequency of multiple resistance; 37 per cent of the fecal coliform isolates were resistant to three or more (three, four, five, six or seven) of the twelve antibiotics.

Grabow et al. (17) indicate that two per cent of coliforms in sewage and sewage polluted water can be expected to carry R factors, and cite the maximum reported incidence of 26 per cent in a hospital effluent. Thus our poultry plant

TABLE 21. SUMMARY, RESISTANCE TO 12 ANTIBIOTICS, FECAL COLIFORM ISOLATES, 1975

ANTIBIOTIC-RESISTANT FECAL COLIFORM ISOLATES			
Resistant to:	PLANT A No. (%)	PLANT B No. (%)	PLANT C No. (%)
TE-50 Tetracycline	44 (55.7)	19 (17.6)	110 (81.5)
PN-25 Ampicillin	25 (31.6)	14 (13.0)	14 (10.4)
KF-25 Cephalothin	4 (5.1)	4 (3.7)	6 (4.4)
CN-10 Gentamycin	0	0	0
K-30 Kanamycin	10 (12.7)	1 (0.9)	43 (31.9)
S-25 Streptomycin	51 (64.6)	26 (24.1)	123 (91.1)
F-200 Nitrofurantoin	4 (5.1)	3 (2.8)	12 (8.9)
PY-100 Carbenicillin	24 (30.4)	13 (12.0)	14 (10.4)
PB-300 Polymyxin B	1 (1.3)	0	0
CT-10 Colistin Sulfate	1 (1.3)	0	0
E-50 Erythromycin	13 (16.5)	10 (9.3)	15 (11.1)
G-300 Sulphafurazole	23 (29.1)	12 (11.1)	17 (12.6)
Total No. of Isolates	79	108	135

TABLE 22. SUMMARY, MULTIPLE ANTIBIOTIC RESISTANCE, FECAL COLIFORM ISOLATES, 1975

Antibiotics Resistant To:	ANTIBIOTIC-RESISTANT FECAL COLIFORM ISOLATES		
	PLANT A No. (%)	PLANT B No. (%)	PLANT C No. (%)
None	12 (15.2)	55 (50.9)	4 (3.0)
One	10 (12.7)	21 (19.4)	21 (15.6)
Two	14 (17.7)	22 (20.4)	45 (33.3)
Three	21 (26.6)	4 (3.7)	37 (27.4)
Four	11 (13.9)	4 (3.7)	14 (10.4)
Five	9 (10.1)	2 (1.9)	9 (6.7)
Six	3 (3.8)	0	4 (3.0)
Seven	0	0	1 (0.7)
Total No. of Isolates	79	108	135

TABLE 23 (a). DRUG RESISTANCE PATTERNS, FECAL COLIFORM ISOLATES

Resistance Pattern							Number Of Isolates		
							Plant A	Plant B	Plant C
TE	PN	KF	K	S	PY	G			1
TE	KF	K	S	F	E				1
TE	KF	F	PB	CT	E		1		
TE	PN	K	S	F	PY				1
TE	PN	K	S	PY	E				2
TE	K	S	F	E	G		1		
TE	PN	S	PY	E	G		1		
PN	S	PY	E	G			1		
TE	K	S	E	G					1
TE	K	S	F	E					1
TE	PN	KF	S	E				1	
TE	PN	S	PY	G			1	1	
TE	PN	S	PY	E			2		1
TE	PN	K	S	PY			4		6
TE	PN	S	PY				4		3
TE	PN	PY	G				1	1	
TE	PN	KF	S					1	
TE	K	S	G				1	1	2
TE	S	E	G				1		2
TE	K	S	E						2
TE	K	S	F						4
TE	S	F	G					1	1
PN	S	PY	G				2		
PN	F	PY	E				1		
PN	S	PY	E				1		
TE	PN	PY					3		
TE	KF	S							3
TE	K	S							18

TABLE 23 (b). DRUG RESISTANCE PATTERNS, FECAL COLIFORM ISOLATES

Resistance Pattern	Number Of Isolates		
	Plant A	Plant B	Plant C
TE K S	2		
TE S F	1	1	1
TE S G	7		10
TE S E	2		4
PN PY E		2	
PN KF S	1	1	
PN S PY	3		
K S G	1		
KF S G	1		
KF K F			1
TE K	1		
TE S	8	8	41
TE F	1		
TE G		4	
PN PY		7	
KF F		1	
K S			3
S E			1
S G	4	1	
PY E		1	
TE Only	2	1	6
KF Only	1		
S Only	4	10	14
F Only			1
PY Only		1	
E Only	2	6	
G Only	1	3	
None of 12 Antibiotics	12	55	4
Totals	79	108	135

TABLE 24 . ANTIBIOTIC ZONE SIZE CATEGORIES, FECAL COLIFORMS AND SALMONELLAE

Antibiotic	ANTIBIOTIC ZONE SIZE CATEGORIES					
	FECAL COLIFORMS			SALMONELLAE		
	No. (%) Susceptible	No. (%) Intermediate	No. (%) Resistant	No. (%) Susceptible	No. (%) Intermediate	No. (%) Resistant
Tetracycline	140 (43.5)	9 (2.8)	173 (53.7)	504 (94.4)	10 (1.9)	20 (3.7)
Ampicillin	268 (83.2)	1 (0.3)	53 (16.5)	534	0	0
Cephalothin	213 (66.1)	95 (29.5)	14 (4.3)	527 (98.7)	6 (1.1)	1 (0.2)
Gentamycin	332	0	0	534	0	0
Kanamycin	250 (77.6)	18 (5.6)	54 (16.8)	339 (63.5)	195 (36.5)	0
Streptomycin	103 (32.0)	19 (5.9)	200 (62.1)	496 (92.9)	27 (5.1)	11 (2.0)
Nitrofurantoin	260 (80.7)	43 (13.4)	19 (5.9)	442 (82.8)	92 (17.2)	0
Carbenicillin	265 (82.3)	6 (1.9)	51 (15.8)	499 (93.4)	35 (6.6)	0
Polymyxin B	321 (99.7)	0	1 (0.3)	533 (99.8)	1 (0.2)	0
Colistin Sulfate	321 (99.7)	0	1 (0.3)	524 (98.1)	10 (1.9)	0
Erythromycin	34 (10.6)	250 (77.6)	38 (11.8)	9 (1.7)	503 (94.2)	22 (4.1)
Sulphafurazole	270 (83.9)	0	52 (16.1)	461 (86.3)	7 (1.3)	66 (12.4)
Total Isolates	322			534		

TABLE 25 . SUMMARY, RESISTANCE TO 12 ANTIBIOTICS, SALMONELLA ISOLATES, 1975

Resistant to:	ANTIBIOTIC-RESISTANT SALMONELLA ISOLATES		
	PLANT A No. (%)	PLANT B No. (%)	PLANT C No. (%)
TE-50 Tetracycline	1 (0.7)	2 (1.4)	17 (6.9)
PN-25 Ampicillin	0	0	0
KF-25 Cephalothin	0	1 (0.7)	0
CN-10 Gentamycin	0	0	0
K-30 Kanamycin	0	0	0
S-25 Streptomycin	2 (1.4)	8 (5.7)	1 (0.4)
F-200 Nitrofurantoin	0	0	0
PY-100 Carbenicillin	0	0	0
PB-300 Polymyxin B	0	0	0
CT-10 Colistin Sulfate	0	0	0
E-50 Erythromycin	10 (6.8)	6 (4.3)	6 (2.4)
G-300 Sulphafurazole	1 (0.7)	40 (28.4)	25 (10.2)
Total No. of Isolates	147	141	246

TABLE 26 . DRUG RESISTANCE PATTERNS, SALMONELLA ISOLATES

Resistance Pattern	Number of Isolates		
	Plant A	Plant B	Plant C
TE S E		1	
KF E G		2	
S E G		1	
TE S		1	
S G		1	
E G		2	2
TE Only	1		17
S Only	2	4	1
E Only	10	1	4
G Only	1	35	23
None of 12 Antibiotics	133	101	199
Totals	147	141	246

effluent data constitute presumptive evidence of a very high incidence of R^+ fecal coliforms in the populations studied.

In spite of this theoretical availability of fecal coliform R factors, salmonellae isolated from the same effluent samples showed much less antibiotic resistance. Only four salmonellae, all from Plant B wastes, were resistant to three antibiotics.

Antibiotics added to some animal feeds in Canada include Chlortetracycline and Oxytetracycline Hydrochloride, Zinc Bacitracin and Bacitracin M, Oleandomycin, Penicillin, Erythromycin and Streptomycin (often in combination with Penicillin).

Antibiotic feed additives thus may account in some degree for the high incidence among fecal coliform isolates of resistance to Tetracycline and Streptomycin, but does not explain frequent resistance to Ampicillin, which was also noted by Vanderpost and Bell (43), Kanamycin, and Sulphafurazole, or the less-frequent resistance to some other test antibiotics. Eleven of the twelve test antibiotics were selected from those considered effective in controlling infections by Gram negative enteric bacteria. Erythromycin is normally considered only for use against Gram positive bacterial infections, but there was a surprisingly low incidence of resistant strains among the isolates.

In summation, poultry plant waste streams appear to offer a considerable theoretical possibility of R factor transfer from resistant coliforms to other enteric bacteria, as discussed by Grabow et al. (17), but there is no evidence that such transfer occurred with respect to salmonellae isolated in the present investigations.

5.6 Salmonella Methodology

A wide variety of enrichment media, selective plating agars and incubation regimes have been advocated for the qualitative detection of salmonellae in water, effluents and other environmental samples. A number of these have previously been used and evaluated by this Laboratory. It is evident that there is no single optimum concentration method, enrichment procedure, selective differential medium or incubation temperature which will ensure the recovery of all Salmonella strains present in any given sample of polluted water. Thus, when any search for salmonellae is planned, alternative procedural options must be considered; the final selection of methods to be employed will inevitably be a compromise between optimum Salmonella recovery and cost and time factors which place a practical limit on the number of replications and the variety of "isolation pathway" tests which can be applied. For these reasons, a number of previously selected isolation procedures were tested in parallel, to provide a basis for the selection of a "minimum, best-option" procedure to be applied to future examinations of poultry plant waste streams.

A modified Moore Swab technique, with sterile gauze pads submerged for 3 to 7 days in water at a sampling site, has been shown by many investigators to be an effective procedure for the concentration and isolation of salmonellae, particularly from moderately-polluted waters. In many case studies, however, sampling schedules at locations remote from the laboratory frequently will not economically permit installation of Moore Swabs at sampling points for more than a few hours. Excessive debris present in raw processing plant wastes, and the severe mechanical effects of rapid flows, agitation and aeration in waste treatment processes, also tend to limit the applicability of the Moore Swab test.

For these reasons, Swab exposure in the present case studies was restricted to 3 - 4 hours. Under these conditions,

Moore Swabs isolated salmonellae from 10 of 13 samples shown to be positive by parallel filtration tests, but tended to be considerably less productive of Salmonella isolates. Previous development work by this Laboratory has shown that filtration of stream and sewage samples through stacked glass-fiber pre-filters, with or without serial filtration of prefilter filtrates through membrane filters, is an effective concentration technique for Salmonella recovery. The prefilter technique has consistently isolated salmonellae from one.l of suspensions containing from 2 to 10 organisms per l. Data presented in Section 5.4 appear to indicate that the filtration test offers a reasonably-simple, reliable procedure for the isolation of salmonellae from poultry plant wastes.

A summary of enrichment broth productivity data, is presented in Table 27, and Tables 28, 29 and 30 show the Salmonella serotype isolation productivity of the three selective plating agars as applied to each of the enrichment broths at the two incubation temperatures used.

Incubation temperatures of 37°, 39°, 41.5° and 43°C have all been used for the isolation of salmonellae; the optimum incubation regime appears to depend on the type and origin of the samples tested and the geographical area studied. Hoadley et al. (20) and other investigators have achieved excellent Salmonella productivity with 37°C incubation. Other workers have found 43°C incubation to be highly selective and productive for salmonellae; this temperature is, however, close to the extinction point for some salmonellae, and the risk of suppression is great unless very precise water bath temperature control can be guaranteed.

In studies of salmonellae in Ontario stream water, we have found 41.5°C to be more Salmonella-productive than 37°C incubation. This was again the case with poultry plant wastes; 41.5°C incubation was consistently more productive of salmonellae than 37°C incubation. This applied to all enrich-

TABLE 27. SALMONELLA ISOLATION PRODUCTIVITY OF DULCITOL SELENITE (DS), SELENITE CYSTINE (CS), AND TETRATHIONATE (T) ENRICHMENT MEDIA, INCUBATED FOR 24 AND 48 HOURS AT 41.5° AND 37°C, 1975.

Plant	No. of Positive Samples	NUMBER OF SAMPLES SALMONELLA-POSITIVE											
		DULCITOL SELENITE				SELENITE CYSTINE				TETRATHIONATE			
		41.5°	37°	24	48	41.5°	37°	24	48	41.5°	37°	24	48
A	Moore S - 3	1	1	0	0	3	3	1	2	3	3	0	1
	Filtered - 3	1	2	0	0	3	3	2	3	3	3	2	1
B	Moore S - 2	1	1	0	0	2	2	1	1	2	2	0	1
	Filtered - 5	0	1	0	0	3	4	2	4	3	2	1	1
C	Moore S - 5	2	3	1	1	4	5	0	2	5	5	1	0
	Filtered - 5	2	3	1	1	5	5	1	2	4	5	1	2

TABLE 28. SALMONELLA ISOLATION PRODUCTIVITY OF PLATING AGARS STREAKED FROM DS, SC AND T ENRICHMENT BROTHS, WITH 41.5° AND 37°C INCUBATION, PLANT A SAMPLES, 1975

Sample Number	Plating Agar	41.5°C INCUBATION			37°C INCUBATION		
		*Serotypes from:			*Serotypes from:		
		DS	SC	T	DS	SC	T
<u>Moore Swab Tests</u>							
1	BG	1	1,3	1	-	-	-
2	BG	-	1	1	-	1	-
3	BG	-	1	1	-	2	-
1	XLD	1	1	-	-	-	-
2	XLD	-	1	-	-	1	-
3	XLD	-	1	1	-	1	-
1	BiS	1	1	1,2	-	-	-
2	BiS	-	1	1	-	-	-
3	BiS	-	1	-	-	1	2
<u>Filtration Tests</u>							
1	BG	1,2	1	1,2,3	-	-	-
2	BG	1	1	1,2,3	-	1	1
3	BG	-	1	1,2	-	1	1,2
1	XLD	1	1	1	-	-	-
2	XLD	2	1	1,3	-	1	-
3	XLD	-	1,2	1	-	1	-
1	BiS	1	1	-	-	1	-
2	BiS	1	1	-	-	1	1,4
3	BiS	-	1	1	-	1	2

* Salmonella Serotypes: 1, S.chester; 2, S.saint paul;
3, S.montevideo; 4, S.infantis.

TABLE 29. SALMONELLA ISOLATION PRODUCTIVITY OF PLATING AGARS STREAKED FROM DS, SC AND T ENRICHMENT BROTHS, WITH 41.5° AND 37°C INCUBATION, PLANT B SAMPLES, 1975

		<u>41.5°C INCUBATION</u>			<u>37°C INCUBATION</u>		
Sample Number	Plating Agar	*Serotypes from:			*Serotypes from:		
		DS	SC	T	DS	SC	T
<u>Moore Swab Tests</u>							
2	BG	-	1, 2	1, 2	-	-	-
3	BG	-	2	3	-	-	-
2	XLD	-	1, 2	1, 2, 3	-	-	2
3	XLD	-	1, 3	2	-	-	-
2	BiS	3	1, 3	2	-	1	-
3	BiS	2	-	3	-	-	-
<u>Filtration Tests</u>							
2	BG	-	-	2	-	-	-
3	BG	3	1, 3, 5	2	-	-	1, 2
4	BG	-	1	-	-	1	-
5	BG	-	-	4	-	-	-
6	BG	-	-	-	-	-	-
2	XLD	-	1, 2	1, 2	-	-	-
3	XLD	3	1	1, 2	-	3, 5	1
4	XLD	-	1	-	-	1, 2, 3	-
5	XLD	-	-	4	-	1	-
6	XLD	-	-	-	-	-	-
2	BiS	-	1, 2, 5	2	-	-	-
3	BiS	-	2, 3, 5	1, 2	-	1	1, 2
4	BiS	-	1	-	-	-	-
5	BiS	-	-	4	-	1	-
6	BiS	-	6	-	-	6	-

*Salmonella Serotypes: 1, S.oranienberg; 2, S.heidelberg;
 3, S.bredeney; 4, S.thompson;
 5, S.saint paul; 6, S.infantis

TABLE 30. SALMONELLA ISOLATION PRODUCTIVITY OF PLATING AGARS STREAKED FROM DS, SC AND T ENRICHMENT BROTHS, WITH 41.5° AND 37° C INCUBATION, PLANT C SAMPLES, 1975

Sample Number	Plating Agar	41.5°C INCUBATION			37°C INCUBATION		
		*Serotypes from:			*Serotypes from:		
		DS	SC	T	DS	SC	T
<u>Moore Swab Tests</u>							
1	BG	1, 2	1, 2, 3	2, 3	-	-	-
2	BG	1	-	1, 2	-	-	-
3	BG	1, 4	2	2	-	-	-
4	BG	-	1	1, 2	-	-	2
5	BG	-	1, 2	2	-	-	-
1	XLD	1, 2	1, 2	-	-	-	-
2	XLD	1	1, 2	1	-	-	-
3	XLD	1	1, 2	-	-	-	-
4	XLD	-	1	1, 2	-	-	-
5	XLD	-	1, 2	1	-	-	-
1	BiS	2	1, 2	2, 3	3	-	-
2	BiS	1	1	1	-	-	-
3	BiS	1	1	2	-	1	-
4	BiS	-	1	-	-	-	-
5	BiS	-	1	3	1	1	-
<u>Filtration Tests</u>							
1	BG	1	1	2	-	-	-
2	BG	1	1, 2, 5	2	-	-	-
3	BG	-	1, 2	1	-	-	-
4	BG	-	1, 2, 3	1, 2	-	1	-
5	BG	-	2, 3	1, 2	-	-	1
1	XLD	1	1	2	-	-	-
2	XLD	1	1, 2	-	-	-	-
3	XLD	-	1, 2, 3	-	-	-	-
4	XLD	-	1, 2, 3	1, 2	-	3	-
5	XLD	-	1, 2	1, 2	-	1, 3	-
1	BiS	1	1	2	-	-	-
2	BiS	1	1	1	-	-	1
3	BiS	-	1	1	1	-	-
4	BiS	4	1, 2	-	1, 3	-	-
5	BiS	-	1, 2	1, 2	-	3	1, 3

*Salmonella Serotypes: 1, S.schwarzengrund; 2, S.manhattan;
 3, S.indiana; 4, S.heidelberg;
 5. S.blockley

ments, but was particularly true for Dulcitol Selenite Enrichment Medium. Thus 41.5°C is clearly the incubation temperature of choice for this type of sample in Ontario, although occasionally some serotypes would have been missed in a few samples without 37°C incubation.

Similarly, no single enrichment medium isolated all of the Salmonella serotypes present in all samples. Selenite Cystine Medium was, however, the most productive; this enrichment isolated the dominant serotype in 56 of 63 sample (Moore Swab and Filtration) trials, as compared to 32 of 63 for Tetrathionate Broth, and 23 of 63 for Dulcitol Selenite Enrichment. Tetrathionate was, however, more productive in finding secondary serotypes in some samples, particularly those from Plant A. The problem of enrichment broth selection is illustrated by the data for Station 3, Plant B, for which Selenite Cystine and Tetrathionate Enrichments consistently isolated different serotypes. For some samples, inconsistent results may be attributable to chance distribution of very low numbers of salmonellae of different serotypes in the waste.

On the basis of these data we would select Selenite Cystine Medium as the enrichment broth of choice, with the proviso that parallel Tetrathionate Broth enrichment would be highly desirable if laboratory work loads permit.

Extending the enrichment incubation time from one to five days with daily streaking of cultures probably would enhance the recovery of all Salmonella serotypes which might be present, but this is not a practical procedure. The data obtained (Table 27) show that increasing the enrichment period from 24 to 48 hours increased Salmonella isolation success, at least marginally. The desirability of longer enrichment (3, 4 or 5) days should be considered, but potential increased productivity must be balanced against the possible disruption of laboratory schedules.

Bismuth Sulphite Agar was included in the trial of plating agars because it has been used very successfully in the isolation of salmonellae from poultry sources in Alberta and because it would grow S. typhi in the unlikely event that this human pathogen was present in poultry plant wastes. While it was reasonably satisfactory in our hands, BiS Agar was less productive than the Brilliant Green and Xylose Lysine Dulcitol Agars applied in parallel. There was some sample to sample variation in the isolation efficiency of BG and XLD Agars; while we would marginally prefer BG Agar, the parallel use of XLD Agar would be highly desirable for effective recovery of salmonellae.

Other relatively non-selective media (Hektoen Enteric Agar and MacConkeys Agar) were rejected on the basis of low productivity and coliform overgrowth interference in previous stream water trials, but other laboratories may find these media useful under different conditions.

In summary, therefore, we propose that future monitor and case studies of Salmonella incidence in poultry processing plant wastes in Ontario include the following test protocols:

1. The concentration of bacteria in sample aliquots of at least 500 ml by the glass fibre prefilter - membrane filter procedure described in this Report. The use of a modified Moore Swab technique should probably be restricted to moderately polluted locations (e.g. receiving streams) where the swabs can be left in place for one to three days;

2. Enrichment of sample aliquots in, (a) Selenite Cystine and (b) Tetrathionate Enrichment Broths, for 48 hrs at 41.5°C;

3. Plating of all enrichment cultures on Brilliant Green and Xylose Lysine Dulcitol Agars, with incubation at 41.5°C for 24 hrs in a water-jacketed incubator;

4. Selection of colonies, representative of all Salmonella suspicious colonies appearing on the selective agar plates, for pure culture study as described in this Report. The presence of salmonellae can be reported on the basis of screening tests and biochemical identification, as described in Sections 4.3 and 5.4. While preliminary serological screening of isolates with polyvalent O Salmonella antisera would be highly desirable for preliminary identification, complete serological characterization of representative isolates to serotype could be deferred until a convenient time; and

5. With this system, reasonably definitive data on Salmonella presence or absence could be available within about 10-12 days of sample collection.

5.7 General Discussion

Major efforts are being made, on a world-wide basis, to find solutions which would significantly reduce the impact of meat and poultry production as a vector of human salmonellosis. In Canada, control measures will be primarily under the aegis of the Departments of Health and Agriculture, and will be aimed at a major reduction in the incidence of product contamination; if real progress is to be made, however, the ancillary problem of recycling salmonellae via waste discharges to the external environment must also be controlled. All control agencies must contribute to the ultimate solution of the salmonellosis problem.

The present study, and the work of Vanderpost and Bell (43) in Alberta, have demonstrated a potential hazard associated with the discharge of raw or partially-treated, undisinfected packing plant wastes to natural waters, which may be used for recreational purposes and for the watering of domestic animals.

More recently, proposals have been made in Alberta for the application of raw packing plant wastes, including manure and blood-line effluents, to crop and pasture lands. Land application may be a tenable solution if the proposal includes a requirement for the immediate plow-down of applied wastes. Other disposal solutions will, however, be necessary during the winter when the land is frozen or snow-covered. Where possible, blood, offal and other solid or semi-solid packing plant wastes should be rendered. In our opinion, spray irrigation should not be considered as an option for the disposal of untreated, undisinfected liquid wastes of animal origin. Such wastes appear to constitute an enteric bacterial infection hazard equivalent to domestic sewage, for which secondary treatment and disinfection is required for land disposal.

During recent years, the dangers inherent in the infection of wild animal and avian populations by salmonellae in waste discharges has become the subject of some concern. The increasing ubiquitousness in the environment of the same serotypes frequently implicated in human salmonellosis has been stressed by a number of investigators. Recent work by Thomason et al. (44) showed that salmonellae were present in 6 of 24 samples of surface water and vegetation from a lush hardwood forest in Georgia, and also in 10 of 76 samples from the top of a granite outcropping. They concluded that even a harsh environment may serve as an ecological niche for the survival and transmission of salmonellae.

On the basis of major investigations of transmission of salmonellae through liquid manure application to land in Sweden, Thunegard (41) concluded that salmonellae, when disseminated in nature, can cause infection and be spread passively in the wild fauna. He also suggested that the marked increase in the occurrence of salmonellae in the wild fauna reported by Hurvell et al. (22) almost certainly had some

connection with the dissemination of manure slurry to land.

For packing plant wastes, effective treatment and disinfection before discharge to water or land will be essential. It is recognized that certain environmental problems can result from the uncontrolled use of chlorine in waste disinfection. In addition to the possible toxic effects on fish and other aquatic organisms, waste chlorination has been shown to result in the formation of at least two groups of organic compounds suspected of being both teratogenic and carcinogenic. Chlorination should be accepted or rejected on a case-by-case basis, depending on whether receiving waters are used for water supply, recreational and other human and domestic animal related purposes. Effective, alternative disinfection procedures which can safely be applied to all waste effluents must be found. Ozonation at present appears to be a possible alternative. Ponding to provide extended storage may be feasible and possibly effective where land is available for this purpose, but exposure of the wild fauna to Salmonella - contaminated ponded wastes could well accentuate the pathogen recycling which we are attempting to eliminate.

Studies designed to evaluate possible waste treatment and disinfection options should thus be conducted to provide a solution to the problem identified. In future studies of this kind, a search for salmonellae should be accompanied by tests for the standard pollution index parameters, and for adventitious pathogens which may be capable of proliferation in the wastes. Nutrient loading variations in waste treatment systems may preferentially stimulate specific bacterial biotypes. The 20°C Standard Plate Count determination is a simple, reliable procedure for the estimation of a group of bacteria which make up a major part of the total aerobic heterotrophic population of a water body. The test may be useful in the general monitoring of bacterial activity and proliferation in a particular treatment process. Recent

attention has been directed to Pseudomonas aeruginosa because of its emergence as a significant human pathogen, and further assessment is needed of this organism's ecological distribution and the role of specific types of fluid environments as reservoirs and/or routes of transmission. Similarly, future assessments could profitably include determination of the densities of Klebsiella pneumoniae, Aeromonas spp, and the Proteus-Providence group in processing plant wastes.

REFERENCES

1. American Public Health Association, Standard Methods for the Examination of Water and Wastewater, Thirteenth Edition, American Public Health Association, New York, (1971).
2. Anderson, E.S., The ecology of transferable drug resistance in the Enterobacteria, Ann. Rev. Microbiol. 22: 131-180, (1968).
3. Anderson, G.G., Fish Inspection Branch, Fisheries and Marine Service, DOE, Personal Communication, (1975).
4. Anonymous, Salmonellosis in Canada, Epidemiological Bulletin, 14: 9, (1970).
5. Anonymous, Foodborne Outbreaks, Annual Summary, 1972, National Center for Disease Control, Atlanta, Georgia, U.S.A., (1972).
6. Anonymous, Industry recommends 50 p.p.m. limit on chlorine for poultry wash, Food Chemical News, pp 26-28, March 23, (1975).
7. Aserkoff, B., Shroeder, S.A. and Brachman, P.S., Salmonellosis in the United States - A five year review, Amer. Jour. Epidemiology, 92: 13, (1970).
8. Baldwin, R.A., The development of transferable resistance in Salmonella and its public health implications, Jour. Amer. Vet. Med. Assoc. 157: 1841, (1970).
9. Cherry, W.B., Hanks, J.B., Thomason, B.M., Murlin, A.M., Biddle, J.W. and Croom, J.M., Salmonellae as an index of pollution of surface waters, Applied Microbiol., 24: 334-340, (1972).

10. Dougherty, T.J., Salmonella contamination in a commercial broiler processing operation, Poultry Science, 53: 814-821, (1974).
11. Dutka, B.J. and Bell, J.B., Isolation of salmonellae from moderately polluted waters, Jour. Water Pollution Control Fed., 45: 316, (1973).
12. Edwards, P.R. and Ewing, W.H., Identification of Enterobacteriaceae, Third Edition, Burgess Publishing Co., Minneapolis, (1972).
13. Ewing, W.H. and Davis, B.R., Media and tests for differentiation of Enterobacteriaceae, National Communicable Disease Centre Publication, U.S. Department HEW, Atlanta, Ga., 22 pp, Jan., (1970).
14. Fair, J.F. and Morrison, S.M., Recovery of bacterial pathogens from high quality surface water, Water Resources Research, 3: 799-803, (1967).
15. Geldreich, E.E., Best, E.C., Kenner, B.A., and Van Donsel, D.J., The bacteriological aspects of stormwater pollution, Jour. Water Pollution Control Fed., 40: 1861-1872, (1968).
16. Geldreich, E.E., Water-borne Pathogens, in Water Pollution Microbiology, Ed. R. Mitchell, Wiley-Interscience, New York, 207-241, (1972).
17. Grabow, W.O.K., Prozesky, O.W., and Smith, L.S., Drug resistant coliforms call for review of water quality standards, Water Research 8: 1-9, (1974).
18. Grunnet, K. and Brest Nielsen, B., Salmonella types isolated from the Gulf of Aarhus compared with types from infected human beings, animals and feed products in Denmark, Applied Microbiol. 18: 985-990, (1969).

19. Harvey, R.W.S., Salmonella contaminated animal feed in relation to animals and man, in Microbiological Safety of Foods, Ed. Hobbs, B.C. and Christian, J.H., Academic Press, London, pp 9-17, (1973).
20. Hoadley, A.W., Kemp, W.M., Firmin, A.C., Smith, G.T. and Schelhorn, P., Salmonellae in the Environment around a Chicken Processing Plant, Applied Microbiol., 27: 848-857, (1974).
21. Hobbs, B.C., Microbiological hazards of meat production, Food Manufacture, 49: 29-30,33-34,54, (1974).
22. Hurvell, B., Borg, K., Gunnarsson, A. and Jevring, J., Studies on Salmonella typhimurium infections in passerine birds in Sweden, The IXth International Congress of Game Biologists, Stockholm, Sept. 3-7, (1973).
23. Lee, J.A., Recent trends in human salmonellosis in England and Wales: the epidemiology of prevalent serotypes other than Salmonella typhimurium, Jour. Hyg. Camb., 72: 185, (1974).
24. Levin, M.A. and Cabelli, V.J., Membrane Filter technique for enumeration of Pseudomonas aeruginosa, Applied Microbiol., 24: 864-870, (1972).

25. May, K.N., Bacterial contamination during cutting and packaging chicken in processing plants and retail stores, Food Technol., 16: 89-91, (1962).
26. Miner, J.R., Fina, L.R., Lipper, R.I. and Funk, J.W., Cattle feedlot runoff - its nature and variation, Jour. Water Pollution Control Fed., 38: 1582, (1966).
27. Miner, J.R. Fina, L.R. and Piatt, C., Salmonella infantis in cattle feedlot runoff, Applied Microbiol., 15: 627-628, (1967).
28. Morris, G.K., McMurray, B.L., Galton, M.M. and Wells, J.G., A study of the dissemination of salmonellosis in a commercial broiler chicken operation, Amer. Jour. Vet. Research, 30: 1413-1421, (1969).
29. Morris, G.K. and Wells, J.G., Salmonella contamination in a poultry-processing plant, Applied Microbiol., 19: 795-799, (1970).
30. Nivas, S.C., Kumar, M.C., York, M.D. and Pomeroy, B.S., Salmonella recovery from three turkey-processing plants in Minnesota, Avian Diseases, 17: 605-616, (1973).
31. Pivnick, H., Programs for the control of salmonella, Proceedings of the Advisory Committee on Epidemiology, Dept. N.H.&W., Ottawa, May 30, (1974).
32. Prost, E. and Riemann, H., Food-borne salmonellosis, Ann. Rev. Microbiol., 21: 405, (1967).
33. Sadler, W.W., Yamamoto, R., Adler, H.E. and Stewart, G.F., Survey of market poultry for salmonella infection, Applied Microbiol., 9: 72-76, (1961).

34. Snoeyenbos, G.H., An approach to identifying and maintaining salmonella-free chickens, Avian Diseases, 15: 28-31, (1971).
35. Spino, D.F., Elevated-temperature technique for the isolation of salmonellae from streams, Applied Microbiol., 14: 591, (1966).
36. Summers, J.L., The sanitary significance of pollution of water by domestic and wild animals - a literature review, Shellfish Sanitation Technical Report, USPHS, 18 pp, (1967).
37. Surkiewicz, B.F., Johnston, R.W., Moran, A.B., and Krumm, G.W., A bacteriological survey of chicken eviscerating plants, Food Technol., 23: 1066-1069, (1969).
38. Taylor, J. and McCoy, J.H., Salmonella and Arizona infections, in Food-Borne Infections and Intoxications, Ed., H. Rieman, Academic Press, (1969).
39. Tennant, A.D., Bastien, J.A.P., Toxopeus, R., Beauchamp, M. and Hayes, J.P., Notes on the isolation of salmonellae from drainage streams, Unpublished MS Report, Environmental Protection Service, Department of the Environment, 15pp, (1974).
40. Thomason, B.M., Biddle, J.W. and Cherry, W.B., Detection of Salmonellae in the Environment, Applied Microbiol., 30: 764-767, (1975).
41. Thunegard, E., On the persistence of bacteria in manure, Acta Veterinaria Scandinavica, 55: 1-86, (1975).

42. Toxopeus, R., Tennant, A.D., Bastien, J.A.P.,
Beauchamp, M., and Hayes, J.P., Notes on
bacterial pollution indicators in a Liquid
manure system, Unpublished MS Report, Environ-
mental Protection Service, Department of the
Environment, 25 pp, (1974).
43. Vanderpost, J.M. and Bell, J.B., A bacteriological
investigation of meat packing plant effluents
in the Province of Alberta, with particular
emphasis on Salmonella, Technology Development
Report EPS 4-NW-75-1, Environmental Protection
Service, Department of the Environment, 87 pp,
(1975).
44. Watanabe, T., Selected methods of genetic study of
episome-mediated drug resistance in bacteria,
Methods in Medical Research, 10: 202, (1964).
45. Wilder, A.N. and MacCready, R.A., Isolation of
salmonella from poultry, New England Jour. Med.,
274: 1453-1460, (1966).
46. Woodburn, M., Incidence of salmonella in dressed
broiler-fryer chickens, Applied Microbiol., 12:
492-495, (1964).
47. Woollen, A., Salmonellosis is an increasing world
problem, Food Manufacture, 3, (1974).

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